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CHEMOTHERAPY OF GAS GANGRENE

By G. B. REED, F.R.S.C.

THE dramatic advance in chemotherapy since the discovery of the sulphonamides in 1936 is now familiar. It will be recalled that when Lister demonstrated that the sepsis of wounds was due to invasion of the tissues by micro-organisms he likewise demonstrated the effectiveness of chemotherapy. From Lister to the present time, search for a perfect antiseptic has been unremitting. Failure to approximate the ideal turned most bacteriologists from chemotherapy to immunity. But with the discovery of sulphonamides and renewed interest in penicillin and other products recovered from moulds and bacteria, chemotherapy has again come into prominence.

I propose to discuss a specialized case: sulphonamides in the prevention of gas gangrene in experimental animals. The data are in part a review of reports published over the last three years and in part based on work in progress. My remarks are addressed to general biologists rather than my immediate colleagues the bacteriologists.

Gas gangrene is primarily an infection in compound fractures and deep lacerated wounds, caused by one or several species of the anaerobic genus *Clostridium*. Some twenty species of the genus are known to occur in infected wounds but three species are most frequently concerned: *C. welchii*, *C. septicum*, *C. novyi*. One, two, or all three species may occur in the same wound, frequently accompanied by one or more additional species of *Clostridia*. Moreover various aerobic organisms such as *Staphylococcus* and *Streptococcus* frequently complicate the picture.

Cases occur with some frequency in peace-time, but gas gangrene is primarily a disease of war. In the British armies in the first year of the last war, some 12 per cent of the wounded were thus infected; in the later years of the war the proportion was reduced to approximately 1 per cent of the wounded. Both the French and the German armies suffered somewhat more heavily. Tetanus, in contrast, developed in some

2½ per cent of our wounded during the first year of the last war and by the end of the war had been reduced by immunization to approximately 0.1 per cent of the wounded.

At the beginning of this war we were in a fortunate position in respect to tetanus. A highly effective antiserum was available and also an efficient toxoid. The situation in respect to gas gangrene, as far as therapy was concerned, had not made much progress since the end of the last war. At this stage it appeared to my colleague John Orr and to me, that sulphonamide therapy might be used, if not curatively, at least to tide a wounded man over from the time of wounding until surgery could be applied. However, considerable evidence had been advanced to show that the sulphonamides act only at a positive oxidation-reduction level and since the gas gangrene organisms are anaerobes and grow only when the surrounding medium is in a negative potential phase it was suggested that sulphonamide therapy could not be effective.

In the hope of obtaining quickly, results of practical applicability, contrary to usual procedure we began with animal experiments and later resorted to test-tube trials. Had we reversed the process we should probably have not gone beyond the test-tube stage, as I shall indicate later.

Gas gangrene was established in guinea pigs by infecting experimental wounds with pure cultures of gas gangrene species or with mixtures of two or more species. Infections from pure cultures of *C. welchii*, *C. septicum*, *C. novyi*, or *C. sordellii*, ten times an average lethal dose, developed rapidly and resulted in 100 per cent fatalities. Mixed infections of two or more of these species somewhat altered the clinical reaction but not the final result. Similarly, the addition of one or more of some sixteen additional species of *Clostridia* to an infection with one of the above-mentioned four species resulted in minor differences in the tissue response but the animals all developed a fatal gas gangrene.

In the first series of trials the infected animals were treated orally with three doses per day of sulphanilamide or sulphapyridine as long as the animals survived. The results were indifferent. There was some prolongation of survival time and a small percentage of recoveries but the difference between treated animals and the untreated controls was too small to be of practical significance.

When sulphonamides are administered orally we know that in one or two hours an approximately equal concentration of the drug is reached in all tissues, including the blood. In infections where normal or increased circulation is maintained in the infected area, or even where

circulation is impaired but blood fluids rapidly diffuse through the infected area, oral administration results in at least as great a drug concentration in the infected area as in other parts of the body. In those infections which tend to early generalization a high blood concentration of drug may be expected to prevent growth of the infecting organism in the blood stream.

Gas gangrene, on the other hand, develops as a massive localized infection of wounded tissue in which the vascular system is frequently impaired. Infection generally spreads rapidly by continuous extension in the tissues, during which soluble toxins are produced in the infected area with a resultant profound systemic toxæmia. Only in the later stages of the disease do the organisms enter the blood stream. This suggested that high blood concentrations of the drug *per se* may be inadequate. What appeared necessary was high concentration in the localized infected tissues—probably higher concentration than could be safely realized by oral or intravenous administration.

We therefore tried the simple and obvious procedure of introducing the drug into the infected tissue by introducing it directly into the wounds. This had previously been tried to a limited degree, with some measure of success, in *Streptococcus* infected wounds in rabbits by Legroux and by Jensen *et al.* in *Staphylococcus* infected wounds in man.

From the point of view of the distribution of the drugs the results were striking. When the drug is administered orally the concentration in the blood and in normal and wounded muscle was found to be essentially the same, as was to be expected from Marshall's work on sulphonamide distribution in the normal body. In sharp contrast, when single doses were introduced into wounds as indicated in the graphs. Fig. 1, the drug concentration in the entire musculature of the wounded leg was more than a hundred times greater than in the musculature of the opposite normal leg or in the circulating blood. It is therefore obvious that by this route the infected or potentially infected tissue receives much the larger concentration of drug.

Results with several thousand guinea pigs infected in experimental wounds with pure cultures of *C. welchii*, *C. septicum*, *C. novyi*, or *C. sordellii*, ten average lethal doses, are briefly summarized in Table I (Reed and Orr, 1941, 1942). Different groups of guinea pigs, 10 to 60 animals in a group, infected with one of the four species were treated with seven different sulphonamides. A corresponding number of infected but untreated control animals was included in each group. In every instance all untreated animals died with characteristic gas

gangrene. The average survival time of these untreated animals infected with *C. welchii* was twenty-five hours and in animals infected with the other three species was from thirty-two to thirty-five hours.

It is apparent from the table that all seven sulphonamides, when applied locally had a marked influence on the survival of animals infected with either of the four principal gas gangrene species. *C. welchii* infections responded most satisfactorily to all the sulphonamides. *C. septicum* and *C. novyi* infections responded somewhat less satisfactorily though the recovery rate with the most efficient drugs was high. *C. sordellii* infections gave a relatively poor response. It is also apparent

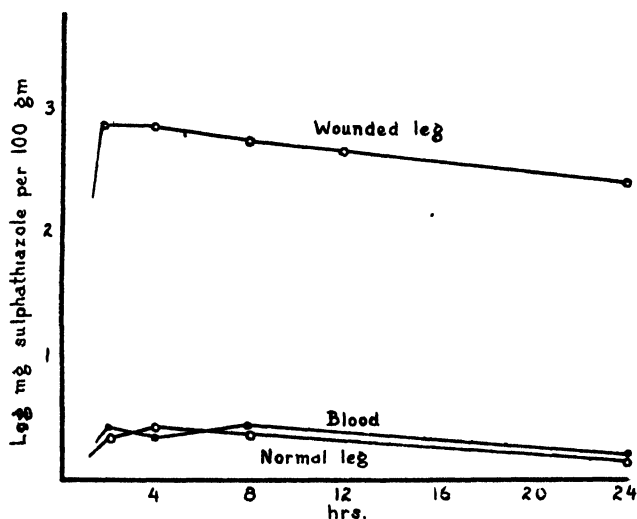


FIGURE 1.—Graphs indicating changing concentration of sulphathiazole in tissues following introduction of single 0.15 gm. doses in an experimental wound in guinea pigs. Ordinates represent log of the concentration of sulphathiazole, mg. per cent; abscissae time in hours after administration.

from the table that among the treated animals which eventually succumbed to the infection the survival time was much greater than in the untreated controls.

Where experimental wounds were infected with two or more species of gas gangrene organisms local sulphonamide treatment was as effective as in the case of pure culture infections with the same species.

It is encouraging to note that *C. welchii* which was present in the last war in approximately 80 per cent of the cases of gas gangrene responds most favourably to sulphonamides. *C. novyi* and *C. septicum*,

which responded slightly less favourably, were present, respectively, in 30 per cent and 13 per cent of the gas gangrene cases. *C. sordellii*, which responds poorly, is a rare organism and has been identified in only a relatively few cases of gas gangrene.

TABLE I

PERCENTAGE RECOVERY OF GUINEA PIGS INFECTED WITH TEN AVERAGE LETHAL DOSES OF PURE CULTURES OF GAS GANGRENE ORGANISMS AND TREATED LOCALLY WITH SULPHONAMIDES

Treatment	<i>C. welchii</i>		<i>C. septicum</i>		<i>C. novyi</i>		<i>C. sordellii</i>	
	No.	Per cent recovery	No.	Per cent recovery	No.	Per cent recovery	No.	Per cent recovery
None	23	0	15	0	15	0
Sulphanilamide	50	41	13	23	48	23	.	.
None	24	0	15	0	15	0		.
Sulphapyridine	63	59	13	55	35	24		
None	20	0	15	0	8	0	10	0
Sulphamethyl-thiazole	43	57	13	78	33	32	8	20
None	10	0	5	0	5	0	5	0
Sulphanilyl-guanidine	10	60	10	75	10	12	10	0
None	10	0	5	0	5	0	10	0
Sulphadiazine	10	80	10	100	10	40	10	20
None	25	0	10	0	10	0	10	0
Sulphathiazole	61	97	20	84	25	84	15	40

It is also evident from Table I that there is a great difference in the effectiveness of the seven sulphonamides tested: sulphanilamide proved to be least, sulphathiazole most effective.

The above data are all from animals treated immediately after infection. From a practical point of view it is of the greatest importance to know how long application of treatment may be delayed. Several groups of guinea pigs were infected and left untreated for two to ten hours. The wounds were then opened and dusted with sulphonamide. Table II indicates results obtained with groups of *C. welchii* infected animals treated at once and at intervals of two to eight hours after infection. Beyond three to four hours, it is seen, the effectiveness decreases but it is also apparent that even after eight hours there is some benefit from the treatment. This period seems very short but it must be remembered that these animals were given a rather massive infecting dose. At three to five hours there was gross evidence of gas gangrene in the wounds. The average survival time of the untreated animals was only twenty-five hours.

Similar results on local sulphonamide therapy have since been obtained by Hawking in England, by Bliss, Long and Smith, and others in the United States. It seems clear that the local application of one of the more efficient sulphonamides at an early stage after wounding will prove to be of value in reducing incidence of, and morbidity from, gas gangrene.

TABLE II

EFFECT OF DELAYING LOCAL SULPHATHIAZOLE TREATMENT OF *C. welchii* INFECTED WOUNDS IN GUINEA PIGS

Interval between inoculation and treatment, hours	No. Animals	Per cent recovered
0	36	95
3	28	70
6	35	60
9	10	5
no treatment	20	0

Once the proposal of introducing relatively large amounts of sulphonamides into injured tissues is raised the old question of toxicity of the bacteriostatic agent for tissues comes into prominence. The cells most concerned in wound healing are fibroblasts and leucocytes. Accordingly tests were made on the influence of sulphonamides on the activity of these two cells (Reed, Orr and Anderson, 1942; Reed and Orr, 1942).

Fibroblast tissue cultures were developed from embryonic guinea pig heart tissue using adult guinea pig serum as the culture media according to the technique of Lumsden. When 50 to 80 mg. per cent of sulphathiazole was added to the serum medium fibroblasts grew at the same rate as in normal serum. When the concentration of sulphathiazole was increased to 100 mg. per cent in the serum medium (approximately saturation) no growth of fibroblasts occurred but after remaining in this concentration for seven days, return to normal serum was followed by the usual rapid outgrowth of fibroblasts.

Blood leucocytes show an approximately similar degree of resistance. In mixtures of leucocytes, serum, killed *Staphylococcus* and sulphathiazole up to concentrations of approximately 80 mg. per cent, phagocytosis proceeded at a normal rate. At higher concentration of sulphathiazole, phagocytosis is inhibited.

It may be expected, therefore, that as long as the fluid in the lumen of the wound remains saturated with sulphathiazole, phagocytosis will not occur and fibroblasts will not grow into the area. A short distance into the tissue where, as shown, the concentration is slightly less than saturation, these cells will behave in a normal manner and as diffusion and elimination reduces the concentration in the lumen, leucocytes and fibroblasts will advance into the area. This is in agreement with the general observation that wounds so treated heal in a normal manner. The one difficulty which presents itself in this respect is where a large excess of the drug is introduced into a small wound cavity. In that case there is a tendency for a cake to form which dissolves very slowly. This is in part eliminated by the use of the drugs in micro-crystalline form.

While the animal results appear to indicate that the problem consists simply in bringing suitable concentrations of sulphonamides to the site of infection, bacteriostatic determinations in the test tube indicate that there are complexities of theoretical as well as practical interest.

Series of such *in vitro* tests have been made in a medium, free from sulphonamide inhibitor, in which all the gas gangrene species grow luxuriantly from a small inoculum. Serial dilutions of the sulphonamide under test were made in this medium. All dilutions were inoculated with a standard inoculum of the organism being tested. The smallest concentration of the sulphonamide which inhibited growth for a three-day incubation period was taken as the end-point. The twenty species of gas gangrene organisms fell into three categories in respect to the amount of sulphonamide required to inhibit growth. The four most important species are distributed through the three categories.

BACTERIOSTATIC CONCENTRATIONS OF SULPHATHIAZOLE

<i>C. sordellii</i> , <i>C. septicum</i>	1 to 2 mg. per cent
<i>C. novyi</i>	5 to 10 mg. per cent
<i>C. welchii</i>	100 to 200 mg. per cent

The most likely explanation of the wide differences in sulphonamide concentration required to inhibit growth appeared to be associated with inhibitor substances. Such substances are now well known to be widely distributed in tissues, tissue fluid and in the bacteria themselves. Indeed the nature of the inhibitor substance has given the best clue to the mode of sulphonamide action.

The hypothesis generally accepted considers that these anti-bacterial substances act by interfering with an essential enzyme of the cell (Lockwood, 1938; McIntosh and Whitby, 1939), or the utilization of an "essential metabolite" of the cell (Fildes, 1940). Woods (1940) found

that para-amino benzoic acid, which in chemical configuration resembles the sulphonamides, acts as a powerful sulphonamide inhibitor. He also demonstrated this substance, and possibly chemically closely related substances, to be the active principle in the naturally occurring sulphonamide inhibitors. If it is assumed that para-amino benzoic acid is

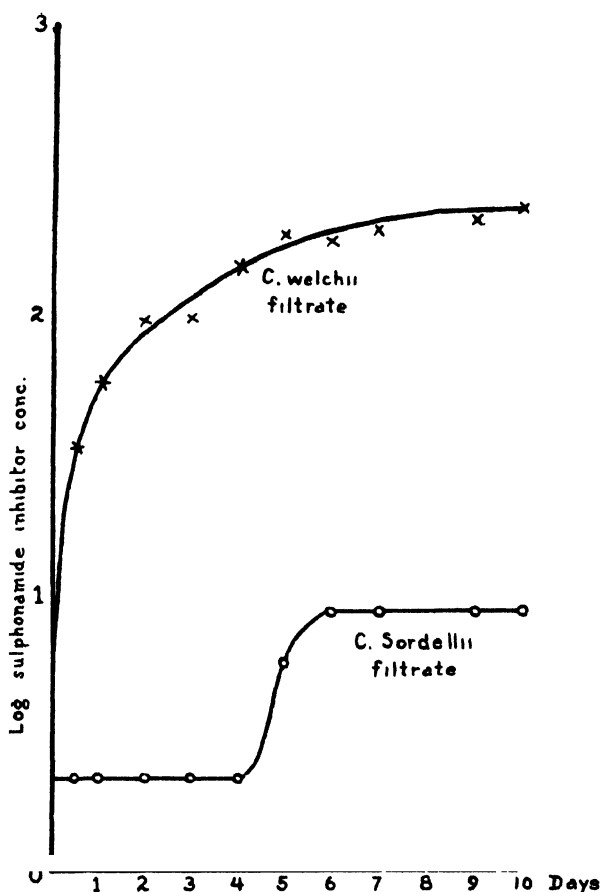


FIGURE 2.—Graphs indicating the rate of production of sulphonamide inhibitor by growing cultures of *C. welchii* and *C. sordellii*. Ordinates represent log of sulphonamide inhibitor concentration; abscissae time in days.

the essential metabolite of Fildes, and there is much evidence that it is an essential cell metabolite, the hypothesis of enzyme interference by the sulphonamides may be enlarged and made specific. It may be suggested that the enzyme mechanism involved in the synthesis of para-

amino benzoic acid is subject to competitive inhibition by sulphonamides.

Assay for para-amino benzoic acid or other inhibitor production by sulphonamide insensitive and sensitive species of *Clostridia* show wide differences. Filtrates of young cultures of *C. welchii* contain some thirty times more inhibitor than filtrates of culture of *C. sordellii* of corresponding ages (Fig. II). Water and ammonia extracts of organisms of the two species show similar differences in inhibitor content, mainly or entirely para-amino benzoic acid.

Or, in terms of the competitive inhibition hypothesis, in the case of the sulphonamide sensitive *C. sordellii*, producing as it does little para-amino benzoic acid, sulphonamide combines competitively with a cell element. Insufficient para-amino benzoic acid is metabolized and no growth occurs. The sulphonamide insensitive *C. welchii* producing much para-amino benzoic acid successfully competes with the sulphonamide; ample para-amino benzoic acid is metabolized and growth occurs.

Inhibitor production then satisfactorily explains the wide differences in *in vitro* bacteriostatic action of closely related species of the gas gangrene group.

It may be noted, however, that species like *C. welchii* which produce large amounts of inhibitor when grown on certain culture media and which are highly insensitive to sulphanilamide in the test tube are satisfactorily controlled in the animal body by sulphonamide therapy. Species like *C. sordellii* which produce little inhibitor and are highly sensitive to *in vitro* bacteriostatic action are controlled in animal tissues with the greatest difficulty by sulphonamide therapy.

Either factors other than sulphonamide inhibitors are concerned or the inhibitor production in living tissues differs widely from the production in sterilized media. A rather detailed analysis of normal animal tissues and tissues in various stages of gas gangrene infection, now in progress, appears to provide part of the explanation of the discrepancy between *in vitro* and *in vivo* bacteriostasis.

Sulphonamide inhibitors, mainly or entirely para-amino benzoic acid, were found in normal tissues. This is in agreement with McLeod's earlier observation. In muscle infected with gas gangrene there is a progressive increase in inhibitor of three to five times the normal content. But in sharp contrast with the production of inhibitor in sterilized media, in living muscle infected with *C. welchii* the rate of increase in inhibitor is approximately the same as the rate of increase in muscle infected with *C. sordellii*. It is not possible to determine the source of the para-amino benzoic acid in these infected tissues. It may be synthesized by the growing organisms or it may be liberated from normal,

dying, or necrotic tissue cells. It is, however, apparent that in culture media inoculated with *C. welchii* or *C. sordellii* the inhibitor production makes conditions much more favourable for sulphonamide bacteriostasis of *C. sordellii* than for *C. welchii*. In the living animal tissues the conditions, due to inhibitor production, are equally favourable for sulphonamide bacteriostasis of these two species.

As far as can be judged from the reaction of guinea pigs heavily infected with gas gangrene, either pure culture, or mixed infection, local sulphonamide therapy, notwithstanding theoretical difficulties, is highly effective. Some of the discrepancies between *in vivo* and *in vitro* bacteriostasis have been resolved by an analysis of inhibitor substances. By the end of the war we should have evidence of the applicability of this form of therapy to human wound infections.

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THE RELATIVE SWEETNESS OF SUCROSE,
GLUCOSE, AND FRUCTOSE¹

By A. T. CAMERON, F.R.S.C.

INTRODUCTION

Sweetness. This primary taste is liable to be modified to a complex sensation through blending with one or more of the others (sourness, saltiness, bitterness), or, more especially, with sensations aroused by stimulation of the nerves of common sensibility, or with smell sensations. The four types of taste bud corresponding to the primary tastes are not uniformly distributed over the mucosa of the tongue. Those sensitive to sweetness or saltiness are most plentiful at the tip, those to sourness along the margins, and those to bitterness towards the base (1).

The relationship of sweetness to chemical constitution has not been satisfactorily determined. Compounds of widely different composition are sweet. These include, for example, (*a*) the ketose, fructose, the aldose, glucose, and their condensed product, sucrose, (*b*) polyhydric alcohols, as glycerol, sorbitol, and dulcitol, and (*c*) numerous unrelated compounds, such as saccharine (orthobenzosulphonimide), dulcine (paraphenetolcarbamide), chloroform, and lead acetate (1).

Marked variation in sensitiveness to sweet compounds and indeed to stimulants of the other tastes is found in different individuals. The extreme example seems to be the reaction of different persons to phenylthiocarbamide (closely related to the sweet dulcine). This tastes bitter to most persons, but to three out of ten it is tasteless (through a hereditary Mendelian recessive factor of taste deficiency) (1).

Earlier work. Measurement of the degree of sweetness of a substance or a solution must be by subjective methods. Comparisons of sweetness are difficult, since one must compare a sensation with the memory of another sensation. It is not surprising that early statements should show marked disagreement. Thus Abel (2) found that glucose

¹In this paper the chemical names for the sugars are used. It is to be regretted that the names employed in commerce and sanctioned by such government publications as the Food and Drug Regulations, almost completely ignore customary scientific terminology. The Canadian use of the term "glucose" for corn syrup is particularly misleading, since the content of the sugar glucose in this syrup is relatively small; even the term "glucose syrup" would be less misleading.

had 40 per cent the sweetness of sucrose, Ayers (3) 80 per cent, Paul (4) 52 per cent, Sale and Skinner (5) 50 per cent, and Biester *et al.* (6) 74 per cent. Sale and Skinner found that fructose had 150 per cent the sweetness of sucrose, Paul 103, and Biester 173 per cent.²

In 1941 an important paper was published by Dahlberg and Penczek, and this, with the two outstanding earlier papers of Paul, and Biester and her colleagues, must be dealt with more fully.

Paul's work was mainly designed to obtain a mixture of saccharine and dulcine which could satisfactorily replace sucrose as far as sweetness was concerned (there being a shortage of beet-sugar in Germany in the early twenties). I have found no detailed description of his procedure, which, however, consisted in tasting two solutions, first in one order, and then in the reverse order. He used twenty to thirty tasters, and found a considerable variation in their findings. Therefore, using a definite concentration of one compound as standard, (i) he determined the two extreme concentrations of a second compound which all his tasters agreed were respectively less sweet, and sweeter than the standard, and (ii) he divided the range between these two into six or eight equal divisions, and compared each of the corresponding concentrations with the standard. Weighting the results, he calculated the concentration of equal sweetness to the standard.

His results for glucose and fructose, using 3 per cent sucrose as standard, have been quoted. He made the important observation that the relative sweetnesses of sucrose, saccharine, and dulcine change with concentration, but denied that this was the case for different sugars, at least for the range 2 to 10 per cent sucrose.

Paul also reported on the phenomenon of enhancement, finding that a solution containing both saccharine and dulcine required relatively less of the two than of either separately to equal in sweetness any standard sucrose solution. For example, he found that the following were equally sweet: 10 per cent sucrose; 0.0535 per cent saccharine; 0.143 per cent dulcine; a solution containing 0.028 per cent saccharine plus 0.012 per cent dulcine.

In the work of Biester and her companions, directed by Willaman (8), an attempt was made to overcome the difficulty of contrasting a sensation with a sensation-memory by ascertaining the thresholds of sweetness-sensation of solutions of the sugars. They used about 30 women-

²It should perhaps be pointed out that Walton's figures (7) in the *International Critical Tables* (1926) are not critical, but merely a compilation of the data then available.

students for each test, and employed the "drop" method. The mouth was washed out with distilled water, and the tip of the tongue dried with an absorbent cotton swab; then a drop of the test solution was placed on the tip from a medicine dropper.

They chose to select the lowest concentration at which 100 per cent of the tasters detected sweetness, and sometimes determined this by extrapolation. They found in this way that 0.75 per cent fructose, 1.30 per cent sucrose, and 1.75 per cent glucose solutions were equally sweet, claiming in consequence that glucose had 74, and fructose 173 per cent the sweetness of sucrose. These values were rather widely accepted until quite recently. It is, however, obvious that if the relative sweetnesses of the sugars do change with concentration (which has proved to be the case) then the values of Biester *et al.* only apply to threshold concentrations. Even then they are not in agreement with later values.

Since they were dealing with threshold sensations, and since, as will be shown, the sensitivity of different individuals varies widely, it may well be argued that they would have obtained a more accurate comparison, had they taken the minimum concentrations at which 50 per cent of their tasters detected sweetness (cf. 15). These, as determined from their data, were 1.25 per cent glucose, 0.625 per cent sucrose, and 0.60 per cent fructose (giving the respective ratios 50, 100, and 104 with sucrose as standard).

Just prior to the work of Dahlberg and Penczek, Renner (9) stated that the relative sweetness of sucrose, glucose, and fructose change with concentration, while Eichelberg (10) reported somewhat similar findings for glucose and sucrose.

Dahlberg and Penczek's paper (11) seems to be the most important and most accurate yet published. The investigators compared sucrose, lactose, maltose, glucose, and fructose, and in addition some corn syrups. They used three to five experienced tasters, and employed the dairy-tasters' procedure of tasting three or more solutions backwards and forwards until these could be arranged in a definite order of sweetness (12). They obtained the following important results:

- (i) The relative sweetnesses of different sugars vary with concentration. Those of maltose, lactose, and glucose, as compared with sucrose, increase with increasing concentration. Glucose and sucrose of 40 per cent or higher concentrations appear to have identical sweetness (cf. Fig. 1).
- (ii) Fructose is always sweeter than sucrose of the same concentration; its relative sweetness increases with increase of concentration (cf. Fig. 1).

- (iii) The sweetness of one sugar is enhanced by the presence in the solution of a second sugar. Thus, if a solution contains sucrose to the extent of 10 per cent, and glucose to the extent of 5.3 per cent, from their curve (reproduced in Fig. 1) it can be calculated that this amount of glucose is equivalent to 3.5 per cent sucrose, so that theoretically the mixture should be equivalent to 13.5 per cent sucrose. Actually it equals 15 per cent sucrose in sweetness, so that the 5.3 per cent glucose in the mixture behaves, as far as sweetness is concerned, as equal to 5 per cent sucrose.

Variations in sensitiveness to sweetness. Several observers have attempted to measure the minimum differences in concentration of the same sugar which can be detected. Sale and Skinner (5) claimed that it was possible, with four observers, to distinguish consistently and accurately between 2.0 and 2.1 per cent sucrose solutions. Dahlberg and Penczek's experienced tasters could distinguish between the following percentage concentrations of sucrose: 2.0 and 2.07; 10.0 and 10.25; 20.0 and 20.5; 30 and 31; 40 and 41.5; 50 and 52.

Biester's inexperienced tasters were less accurate. Over the range from 0.75 to 10 per cent sucrose substantially accurate decisions could be made only if the difference in concentration was at least 1.5 per cent, while there was a considerable variation between different individuals.

It will be seen that my own experience, also with inexperienced tasters, is in closer agreement with that of Biester than of Dahlberg.

PRESENT EXPERIMENTS

Purpose. The experiments were carried out to check the important findings of Dahlberg and Penczek.

Subjects. Groups of medical students from three different curricular years were consecutively tested. The work was carried out slowly, since it could not be allowed to interfere with their studies. This also limited its scope to some extent.

To limit chances of personal error to one person, I made up all solutions, and supervised all tasting experiments, recording results. I took no other part in the experiments, except to ascertain that I myself would have made rather a poor taster.

The first group of students was used for preliminary experiments. The second and third groups were used for accurate measurements.

Material and purity. The following were employed:

Sucrose: (i) "Redpath cubes," purchased in the ordinary way, checked with the polarimeter showed a purity of 98.5 ± 1 per cent. This material was used with the first and second groups. (ii) "B.C. Refinery

pure cane sugar cubes," purchased, showed by the polarimeter 99.5 ± 1 per cent purity. This material was used with the third group for comparison with glucose. (iii) "Redpath cubes" (a later purchase) showed by the polarimeter 99 ± 1 per cent purity. This material was used for the enhancement tests and comparison with fructose.

Glucose: (i) "Dextrosol" (glucose hydrate), a purchased sample, checked by polarimeter to 100.5 ± 1 per cent. This was used with the first group. (ii) "Dextrosol," a gift from the Canada Starch Company, checked by polarimeter to 100 ± 1 per cent. It was used for all subsequent tests.

Fructose: A sample of "levulose" was purchased from the Eastman Company. It gave a slightly yellow coloured solution. Several measurements in the polarimeter indicated only 90.5 ± 1 per cent purity, while quantitative estimation with Benedict's solution indicated 97 per cent. On the assumption that the chief impurity was glucose, these results suggested a composition of 93 per cent fructose, 4 per cent glucose, 3 per cent undetermined.

Since it was believed possible that the alpha- and beta-forms of glucose might exhibit different sweetnesses, all glucose solutions used for comparison with sucrose were made up at least 16 hours before use. In a very few instances solutions were used 40 hours after preparation; none of such solutions showed any cloudiness or other evidence of decomposition due to moulds.

As is, of course, customary in all such experiments, the tasters had no clue as to what sugar they were tasting.

Preliminary experiments. The "drop" method was first used, following Biester's procedure, and rinsing between different solutions, but not drying the tongue, since that only gives it an additional confusing stimulus. It was found that different medicine droppers have different sized drops; furthermore, the same dropper gave drops differing in size according to the rapidity with which pressure was exerted on its rubber bulb. A number of droppers with almost the same calibre were selected, and as far as possible similar pressures were used with them.

The resulting study of tongues showed several varieties, two outstanding. Some subjects could protrude their tongues, so that the ends were like small spoons, holding a drop admirably. Others had a curving end, round which the drop quickly rolled. It was realized that the strength of stimulus almost certainly bore no simple relationship to the area stimulated; I am unaware of anything in the literature which indicates the nature of whatever relationship actually exists.

In these "drop" experiments two solutions were compared, and, half

an hour later, compared again, with the order reversed. The subject was unaware that this was a repetition. In two series of such tests with 25 persons only 3 gave results in both series which were consistent in themselves, and only a slightly larger number gave consistent results in even one series.

Similar tests with spoonfuls of solution, containing measured 5 cc. amounts, gave similar and equally disappointing results.

In both these types of experiments it was found that a fairly large proportion of the tasters always reported that the first solution was the sweeter (persisting first impression), while almost as many always reported that the second solution was sweeter (dominant immediate impression). Hence it was evident that a selection would have to be made of individuals whose taste perception or taste memory was more sensitive than the average. The method of selection will be described later. Of 19 persons so tested 8 were selected.

By this time I had learned from Dahlberg his method of testing, and I employed it in all subsequent tests. Three solutions were usually used, being two different concentrations of one sugar, and a solution of a second sugar. The subject tasted these backwards and forwards as often as he pleased till satisfied that he could arrange them in order of sweetness or that no difference could be detected between two of them. Each subject was informed that two of the solutions were different concentrations of the same sugar and must therefore have a different sweetness, but that the third solution was of a different sugar, and its sweetness might equal one of the others or differ from both.

Dahlberg recommended rinsing the mouth with water in testing dilute, but not concentrated solutions, but my subjects reported that, while rinsing between tests with concentrated solutions (such as 10 per cent sucrose) was definitely confusing, rinsing even between dilute solutions (as 2 per cent sucrose) was of little assistance, and it was gradually omitted, even with these.

In every series of tests, both preliminary and final, some results were obviously wrong. One taster might report, for example, that 12 and 14 per cent sucrose were equally sweet, or even that 12 per cent was sweeter than 14 per cent. Any report including such a false result was completely excluded, while subjects frequently reporting such false findings had all their reports excluded and were not used further.

Time did not permit many accurate measurements with the first group. Those performed indicated:

The sweetness of 2 per cent sucrose lies between those of 3.33 per cent and 4.0 per cent glucose.

The sweetness of 10 per cent sucrose lies between those of 14.3 per cent and 15.4 per cent glucose.

The sweetness of 20 per cent sucrose lies between those of 22 per cent and 26 per cent glucose.

(All the glucose figures, here and subsequently, refer to the compound $C_6H_{12}O_6$, and not to its hydrate.)

Specific tastes of sugars. The various sugars have specific tastes or flavours other than sweetness. Trained tasters can apparently learn to ignore these, and compare their sweetnesses only. But untrained tasters often do not do so. Fortunately the majority do not appear to be able to distinguish these specific flavours, at least as far as sucrose and glucose are concerned, and therefore are not confused by them. However, in the experiments reported in this paper, one or two tasters reported glucose solutions as "bitter," or "sour," or "different in some way," or "tart" and so on, while others reported that weak sucrose solutions, contrasted with very slightly sweeter glucose solutions, tasted "flat." Others claimed to find a marked difference between fructose and sucrose solutions (though this may have been due to impurities in the fructose).

These confusing extra sensations undoubtedly led to a number of the obviously false results which were excluded. They probably caused lesser, non-detectable errors in arrangements of order of sweetness, which account at least partially for the markedly different reports made by different people.

Selection tests. That used with the first group was rather drastic. Two sucrose solutions, 10 and 8.5 per cent, were contrasted; 2 c.c., measured into a spoon from a burette, were taken into the mouth and then spat out after one or two seconds. The mouth was rinsed with distilled water before and after this. In this way each subject tasted three solutions in a particular order, either 10 per cent, 8.5 per cent, 10 per cent, or 8.5 per cent, 10 per cent, 8.5 per cent, the test thus involving: rinse, taste, spit, rinse, taste, spit, rinse, taste, spit, rinse. Half an hour later the test was repeated, with the order reversed (8.5, 10, 8.5, or 10, 8.5, 10). The solutions were all numbered in such fashion that the subjects did not know what they were tasting, nor that the second test was a repetition. They were only informed in each case that the first and third solutions were identical, and were asked to state whether the second was sweeter, equal, or less sweet than the others. Unless they were right in both tests they were eliminated. Only 8 out of 19 reported correctly.

The tests with the second and third groups were of the same nature, but perhaps a little easier, 5 c.c. of solution being employed, and 8 and

10 per cent sucrose compared. About 10 c.c. of tap-water was used for rinsing. In the second group 14 persons out of 30 gave correct results, and in the third only 17 out of 46. These selected persons were used in the tests now to be described. Since a random choice would give a certain proportion of correct results, even this method is not completely

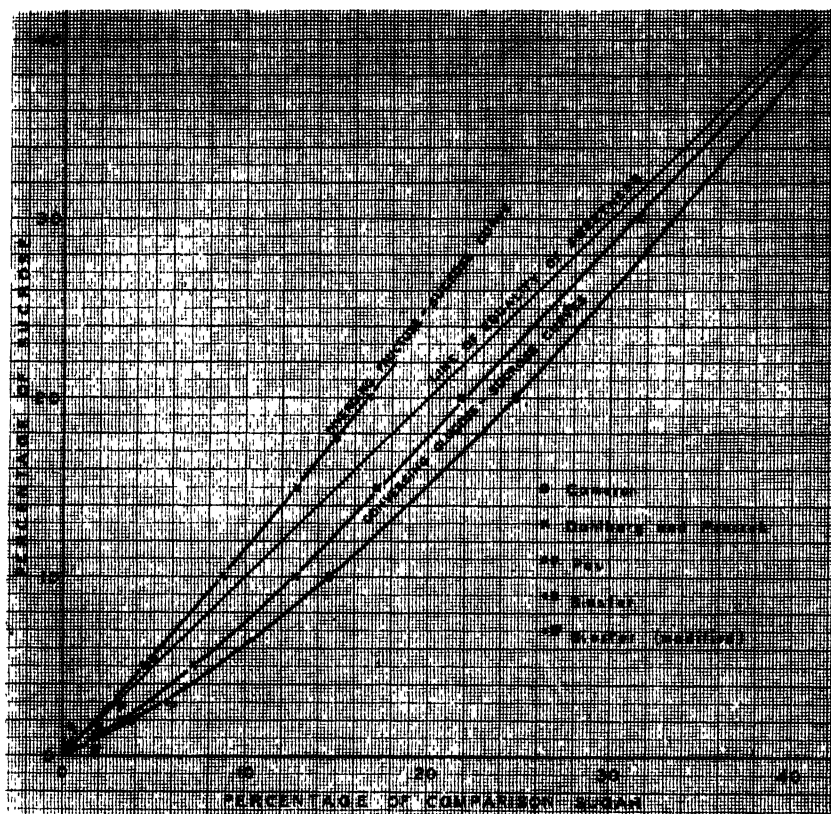


FIGURE 1.—Curves showing comparative sweetnesses of sucrose, glucose, and fructose at different concentrations (from the results of Cameron; Dahlberg and Penczek; Paul; and Biester *et al.*).

adequate, and as has been noted, some of the selected group had subsequently to be eliminated.

Both sexes were tested, and there was no noticeable difference in their relative accuracies. It seems reasonable to conclude that over half the population lack a delicate taste sensation for sweetness.

Accurate comparison of sucrose and glucose. The method employed was a combination of those of Dahlberg and of Paul. Glucose solutions

were found which all tasters agreed were less sweet, and others which all agreed were sweeter than, respectively, 2, 10, and 20 per cent sucrose solutions. Then, in each case, pairs of glucose solutions, within the range of these extremes, and differing sufficiently between themselves that their difference in sweetness should be readily detectable, were compared with the appropriate sucrose solution. A considerable spread of results was obtained. These were weighted, following Paul's procedure. As usual, all solutions were numbered and "scrambled," and only the usual information given (two concentrations of one sugar—so that two solutions must show different sweetness—and one of another sugar). An example will more clearly indicate the method.

COMPARISON OF 2 PER CENT SUCROSE WITH GLUCOSE SOLUTIONS

In the following *S* means per cent sucrose, *G* per cent glucose. The "votes" of those reporting equality were halved between the two solutions concerned, and the resulting totals are shown in brackets

Triads tested: 2*S*/3*G*/3.9*G*; 2*S*/3.3*G*/4.2*G*; 2*S*/3.6*G*/4.5*G*. The results with each triad of solutions gave comparisons between the sucrose solution and each of two concentrations of glucose.

Concentrations compared:	2 <i>S</i> : 3 <i>G</i>	2 <i>S</i> : 3.3 <i>G</i>	2 <i>S</i> : 3.6 <i>G</i>	2 <i>S</i> : 3.9 <i>G</i>
No. reporting <i>S</i> sweeter:	26 . . . (100%)	16 (18) (75%)	11 (12½) (57%)	8 (14) (54%)
No. reporting <i>G</i> sweeter:	0 . . . (0%)	4 (6) (25%)	8 (9½) (43%)	6 (12) (46%)
No. reporting equality:	0	4	3	12
Concentrations compared:	2 <i>S</i> : 4.2 <i>G</i>	2 <i>S</i> : 4.5 <i>G</i>	2 <i>S</i> : 4.8 <i>G</i>	
No. reporting <i>S</i> sweeter:	3 (3½) (15%)	2 . . (9%)	0 . . . (0%)	
No. reporting <i>G</i> sweeter:	20 (20½) (85%)	21 . (91%)	23 (100%)	
No. reporting equality:	1	0	0	

Calculation:

	(A) Glucose concentration per cent	(B) Percentage reporting sucrose sweeter	(A) × (B)	100 <i>A</i> or	(B) Percentage reporting glucose sweeter	(.1) × (B)	100 <i>A</i>
	3.0	100	300	300	0	0	300
	3.3	75	247.5	330	25	82.5	330
	3.6	57	205.2	360	43	154.8	360
	3.9	54	210.6	390	46	179.4	390
	4.2	15	63	420	85	357	420
	4.5	9	40.5	450	91	409.5	450
	4.8	0	0	480	100	480	480
Extreme difference	1.8	Totals	1,066.8	2,730		1,663.2	2,730

$$(1,066.8 \times 1.8)/2,730 = 0.70 \quad (1,663.2 \times 1.8)/2,730 = 1.10$$

$$3.0\% + 0.7\% = 3.7\% \quad 4.8\% - 1.1\% = 3.7\%$$

Conclusion: 2.0 per cent sucrose and 3.7 per cent glucose have equal sweetness.

No attempt has been made to calculate the probable error in these tests. Obviously such results can only be considered as approximating to the true figures for equality and suggest that it would be unwise to stress any particular figure too definitely. The larger the number of such *untrained* tasters the greater will be the degree of probable accuracy. This is illustrated by considering the results of the above comparison as found by the second and third groups separately. Those for 11 persons of the second group gave the figure 3.5 per cent glucose, and for 15 persons of the third group the figure 3.8 per cent.

The complete results for the three different concentrations of sucrose are shown in the following Table, in which the extreme concentrations of glucose represent in each case that which all found sweeter and that which all found less sweet than the corresponding sucrose solution.

TABLE I

Sucrose solution Percent	Extreme concen- trations of glucose Percent	Minimum difference of glucose concen- tration Percent	Minimum difference of glucose concn. in each triad Percent	Number of tasters	Glucose solution of equal sweetness Per cent	Ratio S : G	Dahlberg's Ratio S : G
2.0	3.0- 4.8	0.3	0.9	22-26	3.7	1 : 1.85	1 : 1.6
10.0	13.0-16.5	0.5	1.5	22-23	14.6	1 : 1.46	1 : 1.27
20.0	20.0-27.5	1.5	3.0	15-18	24.8	1 : 1.24	1 : 1.09

Though the actual figures differ somewhat from those of Dahlberg (cf. also Fig. 1), the results confirm his important conclusion that relative sweetness varies with concentration. Possible causes of the actual differences in the figures will be considered later.

The relative sweetness of alpha- and beta-dextro-glucose. The marked sweetness of such polyhydric alcohols as sorbitol and dulcitol must be associated with their CHOH groups, and it seems intrinsically probable that the spacial arrangement of these groups may affect sweetness. The sweetnesses of these two alcohols, according to Paul (4) do actually differ by about 20 per cent; recent measurements are lacking.

There seems therefore good probability that the different spacial position of one of the CHOH groups in alpha- and beta-glucose may cause a sweetness difference between these two forms. Sweetness tests have been made at three different times, using 14 tasters in all. In these a 16-hours old solution of glucose (equilibrium mixture of alpha- and

beta-forms) was compared with one just made up. Dr. F. D. White was good enough to make polarimetric measurements of the latter solution simultaneously, so that the proportion of alpha-glucose present at the time of sweetness-comparison could be calculated. The following results were obtained.

TABLE II

Old solution	New solution	No. of tasters reporting		
Proportion of alpha-glucose present Per cent	Proportion of alpha-glucose present Per cent	(1) New solution sweeter	(2) Old solution sweeter	(3) Equality
37	89-85	6	1	1
37	83-78	4	1	1
	Total	10	2	2

Judging by the general results with the tasters used by me, the large majority reporting the fresh solution sweeter can be regarded as reasonable evidence that this is actually sweeter and therefore that alpha-glucose is sweeter than beta-glucose. It would not be easy to measure the relative sweetnesses of the two accurately, but similar majority findings in the sucrose-glucose comparisons suggest that the fresh 10 per cent glucose solution may well have a sweetness equal to at least 10.5 per cent equilibrium solution.

Enhancement effect. Time permitted only a single experiment, with 7 tasters. The following triads were tested: (a) 15S, (10S + 4.5G), (10S + 6.5G); (b) 15S, (10S + 5.5G), (10S + 7.5G). The results and their evaluation follow:

Concentrations compared	15S: (10S + 4.5G)	15S: (10S + 5.5G)	15S: (10S + 6.5G)
No. reporting sucrose sweeter	7 . . . (100%)	4 . . . (57%)	0 (½) (7%)
No. reporting mixture sweeter	0 . . . (0%)	3 . . . (43%)	6 (6½) (93%)
No. reporting equality	0	0	1

Concentrations compared . . .	15S: (10S + 7.5G)
No. reporting sucrose sweeter . .	0 . . . (0%)
No. reporting glucose sweeter	7 . . . (100%)
No. reporting equality	0

Calculation:

		(A)	(B)		
		Concentration of added glucose	Percentage reporting sucrose sweeter	AB	100A
		4.5	100	450	450
		5.5	57	313.5	550
		6.5	7	45.5	650
		7.5	0	0	750
Extreme difference	3.0			Totals	809 2,400
		(809 × 3)/2,400 = 1.0			
		Whence concentration of added glucose to give equal sweetness			
		4.5 + 1.0 = 5.5%			

The added 5.5 per cent of glucose can therefore replace, as far as sweetness is concerned, 5.0 per cent of sucrose, a ratio of 1.1 to 1.0, whereas, from the results in this paper, the curve in Fig. 1 indicates that 5.5 per cent glucose is only equal to 3.2 per cent sucrose. While the fewness of the observations does not permit stress to be laid on the actual figures, they are in close agreement with Dahlberg's already quoted, and do suffice to confirm the existence of this enhancement effect for glucose and fructose, which, it will be remembered, parallels that observed by Paul for saccharine and dulcine.

Relative sweetness of fructose. It seems of some importance that this should be determined accurately. It has been mentioned that Dahlberg's results show that fructose becomes relatively more sweet, as contrasted with sucrose, with increasing concentration (cf. also Fig. 1), and this appears to be the only example of this nature. With all other sugars sweetnesses tend towards equality with increasing concentration, which might be explained on the basis of sense-saturation. Results with fructose cannot be explained in this way.

Although the sample of fructose available was not very pure, it was thought that at least some approximate results might be obtained with it. Seven tasters were used, of a small group which had given the most consistent results.

A comparison of 5 per cent sucrose with 4.0, 4.4, 4.8, and 5.2 per cent fructose (uncorrected for impurities) by the usual method indicated that 4.6 per cent fructose was equally sweet. A comparison of 5 per cent fructose with 4.0, 4.8, 5.6, and 6.4 per cent sucrose, indicated that 5.4 per cent sucrose was equally sweet.

Comparison of 15 per cent fructose with 15.25, 16.0, 16.75, 17.5, 18.25, 19.0, and 19.75 per cent sucrose indicated that 17.7 per cent sucrose was of equal sweetness.

These values lie very close to Dahlberg's sucrose-fructose curve (cf. Fig. 1) and show the same divergence with increasing concentration. However, the tasters showed difficulty in excluding the specific taste or flavour of fructose (or of impurities in it), and this probably affected their conclusions to some extent. In spite, therefore, of the fair agreement with Dahlberg's figures, it is to be hoped that a further comparison of sucrose and fructose will be made by someone who has at his disposal an adequate amount of absolutely pure fructose.

DISCUSSION

The differences between the actual figures found by Dahlberg and Penczek and those now reported are attributable to two possible causes: (i) If these observers used freshly prepared glucose solutions, these would be relatively slightly sweeter than the corresponding equilibrium solutions used in this research. (ii) The less experienced tasters used by me were probably more influenced by the specific flavours of the sugars, and to that extent my figures may be less accurate; it has already been stated that they should only be regarded as approximate.

The anomalous position of fructose has been dealt with.

Both Paul and Dahlberg have attempted to explain the apparent enhancement effect when two sweetening agents are mixed, the former as in reality due to a summation effect, the latter as due to different rates of physiological stimulation by the different sugars. The following analysis of results suggests an explanation akin to that of Paul.

In this research it was found that 10 per cent sucrose and 14.6 per cent glucose were equally sweet. Also that if 5.5 per cent glucose was added to 10 per cent sucrose, the mixture was equal in sweetness to 15 per cent sucrose. If 5.5 and 14.6 per cent glucose are added, to give 20.1 per cent glucose, this, from Fig. 1 (using the curve for my data), is equal in sweetness to 15.2 per cent sucrose, or, within the limit of error, to 15 per cent sucrose.

Dahlberg's results (11), subjected to the same treatment, and using the smoothed curve in Fig. 1 obtained from his own values, are shown in Table III.

TABLE III

(1) Equivalent sweetnesses	(2) Mixture calculated in terms of glucose	(3) Calculated equivalence of (2) in terms of sucrose
15S and (10.0S + 5.3G)	12.7G + 5.3G = 18.0G	15.7S
25S and (16.7S + 8.3G)	19.0G + 8.3G = 27.3G	25.6S
40S and (26.7S + 13.0G)	28.2G + 13.0G = 41.2G	40.6S

The agreement between the actual and calculated sucrose values is within the limit of error for the two higher values, and but little outside it for the 15 per cent sucrose.

This suggests that the phenomenon is related to the converging degrees of sweetness of glucose and sucrose with increasing concentration. Further that, perhaps because of a taste-saturation effect, when for example 5, 10, and 15 gm. of sucrose are respectively dissolved to make 100 c.c. of solution, the sweetness of the 15 per cent solution is not equal to the sum of those of the two weaker solutions, but is a lesser "quantity." In dilute solutions, each molecule of a sugar is more efficient as a taste stimulator. The increment of sweetness which any specific weight of sucrose or glucose adds to a solution is in part determined by the sweetness already possessed by that solution. These views are exemplified by the diagrams in Fig. 2, in which *heights* are proportional to sweetness. In this figure equal heights are based on the

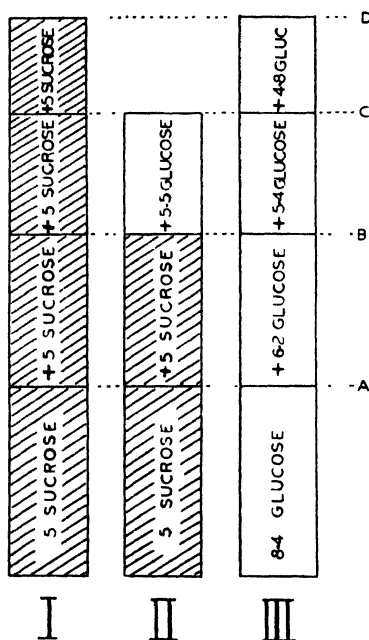


FIGURE 2.—Comparison of successive increments of sweetness produced by sucrose and glucose. Figures refer to grammes of sugar per 100 c.c. of solution. Sweetness is proportional to height. *A*, sweetness of 5 per cent sucrose, *B*, of 10 per cent, *C*, of 15 per cent, and *D*, of 20 per cent sucrose. *I*, effect of successive additions of sucrose. *II*, results from "enhancement" experiment. *III*, results from actual comparisons of sucrose and glucose, and the curve in Fig. 1.

experimental data, but the ratios between the heights of successive increments are assumed.

If these views are strictly applied, then it follows that at still higher concentrations increments of sweetness produced by additions of specific amounts of sucrose will be equally produced by addition of *smaller* amounts of glucose, and some of Dahlberg's results actually suggest that this is the case (cf. Table III). It is to be noted, however, that the underlying hypothesis should apply to all sugars, and the available data for fructose do not appear to fit it. Nor does it apply with sucrose and glucose unless the heavier-moleculcd sucrose is assumed to produce the initial effect (and this assumption agrees with Dahlberg's explanation of the phenomenon). Further experimental work seems desirable to test it, and to explain thoroughly this apparent anomaly of enhancement.

Whatever it be due to, the effect (termed by Dahlberg and Penczek the supplemental action of sugars) is of considerable practical importance, and applies to corn syrups as well. They considered it noteworthy that a high conversion (i.e., enzyme-converted) corn syrup could replace sucrose pound for pound in a 25 per cent solution without affecting sweetness, while even ordinary corn syrup was, for replacement purposes, as sweet as sucrose in a 40 per cent sucrose solution (11).

The present regulations under the Food and Drugs Act of Canada (13) place sucrose in a preferred position. (i) It is defined as synonymous with "sugar," while other sugars require an additional description, as "anhydrous starch sugar" for dextrose (i.e., glucose). (ii) Any replacement of part of the sucrose content of a processed food by a second sugar calls for special labelling, of a type to suggest substitution; substitution always suggests a less desirable product. (iii) In certain food materials such as jams, jellies, and mince-meat, the use of any other sugar except sucrose as sweetening agent is prohibited.

By Order in Council of May 21, 1942, under the authority of the War Measures Act (14) it is made virtually compulsory to replace 25 per cent of the sucrose used in all such foods (processed fruits, vegetables, and ice-cream products) by the same amount of glucose or corn syrup, without label declaration.

It is doubtful if the general public has become aware of this war-time regulation, and one may conclude that no especial difference in sweetness of products is observable from such replacement since it came into force. The results of Dahlberg, confirmed by those now reported, show, in fact, that such a replacement scarcely affects the degree of sweetness of moderately concentrated solutions, and certainly not to an extent detectable by the average individual.

It would seem, therefore, that as far as sweetness is concerned the war-time regulation is also justifiable for peace-time conditions, at least as regards the permissive use of glucose for partial replacement without any label declaration which might suggest substitution and inferiority.

The results of this paper, and those of Dahlberg, bear upon the relative sweetness of sucrose and the invert sugar formed from it by hydrolysis (such inversion occurs to a considerable extent in the canning of fruits, etc.). Early observations show no agreement, there being about as many claims that inversion lessens sweetness as that it causes an increase. Willaman (8) has summarized this early literature. His own conclusions from determination of threshold values by use of the drop method are of little value. Calculations from Dahlberg's figures for glucose and fructose suggest that inversion should lessen the sweetness of dilute sucrose solutions, but that at higher concentrations (10-20 per cent) the sweetness will be but little affected, and at still higher concentrations may even be slightly increased. If the enhancement effect applies, as it should, then inversion will increase sweetness at somewhat lower concentrations than otherwise.

ACKNOWLEDGEMENTS

Thanks are due to Dr. F. D. White for carrying out all the polarimetric measurements recorded in this paper, and to the Canada Starch Company for a gift of "dextrosol."

Thanks are also due to the large number of students who patiently allowed themselves to be used as tasters throughout the experiments.

SUMMARY

The literature dealing with the sweetness of sugars is reviewed.

It is shown that the majority of people lack a delicate taste sensation for sweetness.

Confirming the work of Dahlberg and Penczek, it is shown that the relative sweetnesses of different sugars vary with concentration and also that the sweetness of one sugar is apparently enhanced by the presence of a second sugar. An explanation of the latter phenomenon is offered.

Evidence is given that alpha-dextro-glucose is sweeter than beta-dextro-glucose.

The practical implications of these results are discussed.

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RELATIONSHIP IN NORTH AMERICAN FAMILIES OF
GYMNOBLASTIC HYDROIDS

By C. McLEAN FRASER, F.R.S.C.

ON account of the very nature of marine gymnoblastic hydroids the family relationship has not received the attention it deserves.

With the intertidal, or very shallow water species, the situation is not so serious because the specimens can readily be taken alive and uninjured, but many of them are so small that they are apt to be overlooked by anyone but an experienced observer.

With the species from deeper water, the difficulties in getting live, uninjured specimens are great. With few exceptions hydroids live on rocky or shelly bottom, and if they are collected by dredge, trawl, or tangle, some of the rocks or shells are invariably caught up as well, and hence the whole contents are subject to unavoidable rough usage. The few that live in muddy bottom or in the sand are not much better off for the weight of the mud or sand has a crushing effect. The calyptoblastic hydroids are fairly well protected by the presence of skeletal structures, and even the hydranths and gonophores are protected. The gymnoblastic hydroids, on the other hand, have few or no protective structures even in the main part of the colony, and none whatever for the hydranths and gonophores. In consequence, many of the specimens are macerated beyond recognition when they reach the deck or the sieves. Other specimens are injured badly enough that unless they can be preserved or examined almost immediately, they disintegrate before they can receive attention. Unless the collector is paying special attention to hydroids, this attention can seldom be given. Probably this accounts for the fact that there are relatively so few gymnoblastic species in general, marine, zoological collections.

Even with the information available, it would be of interest to follow each individual character through the North American families of these gymnoblastic hydroids as a study in the relationship and evolutionary development. To serve for a beginning for such a study a discussion of the nature and the arrangement of the tentacles may be appropriate, as the tentacles are nearly always present and the arrangements of these follow quite well-defined lines.

As a basis for discussion it would seem fair to assume that the fili-form tentacles are more primitive, less specialized, than the capitate ten-

tacles, and that the scattered arrangement is more primitive than the more orderly arrangement.

On both of these counts the family *Clavidae* qualifies as the most primitive family of the North American, marine, gymnoblastic hydroids. In every species the filiform tentacles show little or no variation in size, and in all the genera, the scattering is complete. In no case are the gonophores developed directly in relation to any hydranth or hydranths.

From the promiscuous distribution of the tentacles in this family an orderly arrangement has evolved along two different lines, neither of which may have evolved from the other but both of them directly from the scattered condition. In the one instance, a single whorl or verticil of tentacles appears around the base of the hydranth, and in the other, one whorl appears around the base and one or more whorls around the mouth, at no great distance from it. The two are known as the proximal or basal series and the distal or oral series.

In the first of these groups, five families are included. Among these, two of them, the *Atractylidae* and the *Eudendridae*, are less specialized than the others, as far as the tentacles are concerned, and they differ little from each other. In the *Atractylidae*, however, in all of the genera except *Dicoryne*, the gonophores are not developed in relation to any hydranth, and there is no definite difference in appearance between the male and the female colonies. In *Dicoryne*, the gonophores appear at the base of an aborted hydranth, devoid of tentacles.

In the *Eudendridae*, there is usually evident sexual dimorphism and the gonophores are associated with hydranths that show a varying degree of abortion in the different species or even in the different sexes of the same species.

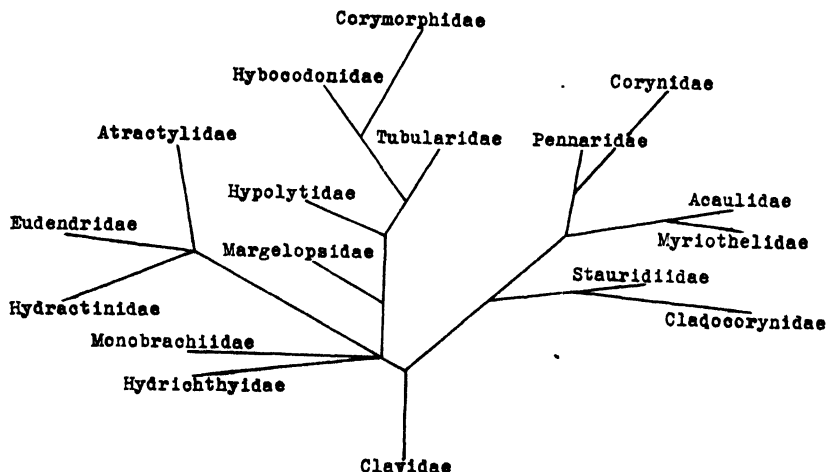
The family *Hydractinidae* shows its specialization in its tendency to polymorphism, which is indicated to some extent in the number more than in the arrangement of the tentacles. The variation in the number of different forms of zooids appears to be a specific rather than a family character. In the nutritive zooids throughout, the whorl of tentacles is similar to that in the families already mentioned, but in the generative zooids there is almost always a reduction of the number and the size of the tentacles as compared with the nutritive zooids, these varying until they may be entirely absent. On the offensive and sensory zooids that develop in some of the species, no tentacles develop.

In the *Monobrachidae*, specialization takes place in quite a different way. The whorl, if one can call it such, consists of a single tentacle, but the lack in number is compensated for by the length, mobility, and gen-

eral activity of the single tentacle, and by the presence of such numerous batteries of nematocysts, that possibly outnumber those on the complete whorl of tentacles in many species of the other families.

In the *Hydrichthyidae* there is retrogressive evolution. As the species belonging to this family are parasitic on hosts that are continuously available for a food supply, there is no necessity for tentacles, and none is developed. Its other characters associate it with this group.

When the tentacles appear in basal and oral whorls, except in one family, the two whorls are quite unlike in the length and the mobility of the tentacles, and usually in the number as well. The exception appears in the *Margelopsidae*, a family of pelagic species, where the free-



Genealogical Tree of the North American families of Gymnoblasic Hydroids as indicated by the nature and the arrangement of the tentacles.

swimming life of the individual zooids precludes any benefit to be derived from differentiation that appears in the other families. Here the tentacles in all of the whorls are similar, and the whorls have a similar number, which is never large.

In all the other families of this group, the numerous basal tentacles appear in a single whorl and they are long, slender, and active, especially suitable for making contact with prey over a relatively wide area. The shorter, oral tentacles, well adapted to put the finishing touches on the killing or paralyzing the live food and getting it into the mouth, provide practically all of the differentiation. In all of the species of this group, the gonophores are associated with the body of the main, nutritive

hydranth, and in no case, with separate hydranths, aborted or otherwise.

The simplest arrangement of these oral tentacles appears in the *Tubularidae* and in the *Hypolytidae*, where they are arranged in a single whorl. The differences in size and number indicate specific rather than family characters. There is no constant difference in them in the two families.

In the *Hybocodonidae* the oral tentacles appear in two whorls, quite closely placed, with the tentacles in the proximal whorl longer than those in the distal, so that the tips of the tentacles in the two whorls, when the tentacles are extended, are at much the same level. There is nothing to indicate that this arrangement represents a stage in the change-over from the scattered condition to that of the tentacles in a single whorl. It is much more likely to be a secondary development from the single whorl condition, to allow for an increase in the number of tentacles without reducing the effectiveness of each.

This process of whorl overlapping is carried to the extreme in the *Corymorphidae*, where there are several whorls in succession, each overlapping the one immediately distal to it.

In the remainder of the families, the filiform tentacles are replaced entirely, or in part, by capitate tentacles. Capitate tentacles in general are not so mobile as the filiform tentacles, but their paralysing power is much increased because of the presence of powerful, closely-placed batteries of large nematocysts in the terminal knob or cap.

In the two families, the *Pennaridae* and the *Acaulidae*, that have retained the filiform tentacles, or some of them, they appear as a basal whorl, similar to that in the *Tubularidae*. In the *Pennaridae*, the capitate tentacles are quite long and slender and the terminal cap is very definite. They are loosely scattered over the body of the hydranth, but there is some indication of a verticillate arrangement. In the *Acaulidae*, the capitate tentacles are shorter, but they are much more numerous and more closely crowded on the body of the hydranth. It can scarcely be said that the one of these is more specialized than the other, because the nature and the arrangement of the tentacles in each case are well suited to the conditions in which they live. In the *Pennaridae*, the species grow in branched colonies, attached to the rock or other hard surface on rocky bottom, hence the colony can sway enough to make considerable movement in the hydranth possible, and the long, scattered tentacles are effective. On the other hand, the species of *Acaulidae* remain as individuals, with only a rudimentary pedicel or stem. enough to hold them in position in the soft bottom. So little movement of the hydranth is possible that it is necessary to have effective means of attack on a food

supply coming anywhere near, from any direction.

Among the families in which no filiform tentacles are developed, there are two that parallel the families, *Pennaridae* and *Acaulidae*, as far as the capitate tentacles are concerned, with a similar degree of fitness in each case. In the *Corynidae* there is a similarity to the *Pennaridae*, and in the *Myriothelidae* to the *Acaulidae*.

In the *Stauridiidae*, there is a marked specialization in the tentacle situation. The number of tentacles is much reduced. They appear in one or more whorls. (In the only genus represented in North American waters, *Cladonema*, there is but one oral whorl with but four tentacles in the whorl.) To offset the reduction in number, the tentacles are relatively long and very actively mobile; the batteries of nematocysts are closely packed in the terminal cap, and also are present, although not so closely packed, throughout the length of the tentacle. Besides these tentacles, that may be considered normal, near the base of the hydranth, or possibly below it (it is difficult to say where the hydranth body joins the pedicel or stem) there is a whorl of four false tentacles, rigid and without terminal enlargement. In some species they appear to be definite tactile organs.

Finally in the *Cladocorynidae* there appears something quite new, unique among hydroids. The whorl of capitate tentacles around the mouth makes connection with some of the other families mentioned, but over the remainder of the body of the hydranth, appearing in whorls, are branched tentacles, with each of the small branches capitate. Although the family is not far removed from some of the genera of the *Stauridiidae*, it provides the ultimate in tentacle specialization.

In the families reported from the North American waters, a large area, but limited as compared with the inhabitable area for the growth of hydroids in all quarters of the globe, it is evident that evolution in the nature and the arrangement of the tentacles has provided a number of series that spread out as they proceed from the primitive type to fill a wide variety of ecological niches. If the families in which representatives have not been reported from this area, but have been reported from other waters of the globe, were included in the examination, the picture would be even more striking.

If other characters were considered in the same way, it is probable that there would be a high degree of parallelism in the series indicated, since variation in the one character is almost sure to be accompanied by variation in other characters as well. Because of this it may be worth while to indicate relationship in these gymnoblastic families by a genealogical tree.

A SYSTEMATIC STUDY OF THE MAIN ARTERIES
IN THE REGION OF THE HEART—AVES VIICORACIIFORMES—PART 1¹By FRED H. GLENNY²

Presented by E. HORNE CRAIGIE, F.R.S.C.

ABSTRACT

Thirty-eight species of birds, representing eight families of the order Coraciiformes, were dissected and diagrams of the arrangements of the main arteries in the neck and thorax prepared. Essential differences in arrangement-patterns within the different families were noted. In two families, both bicarotidinae normales and laevo-carotidinae were observed. Fundamental differences in family patterns were found chiefly in the relative positions (origin from the subclavian artery) of the coracoid major, axillary, and intercostal arteries, and secondarily by the presence of both internal carotids or presence of the left internal carotid alone within the hypapophysial canal. Presence of the ligamentum aortae and right ligamentum botalli was noted for each species studied. Certain embryonic vessels become functionally modified in the adult. The right radix aortae may serve as a dorsal intercostal artery in certain species of Alcedinidae, may become occluded and remain as a ligamentous vestige, or may become completely atrophied and disappear.

INTRODUCTION

IN several recent papers on the main arteries in the region of the neck and thorax of birds, the writer has tried to present the basic arrangement-patterns of various Orders of birds, as well as the more important family characteristics and differences, in so far as this was possible within the limitation of numbers of species, specimens of any one species, and time in which to make adequate studies on any one group of birds—along with the difficulties encountered in obtaining many species of birds.

Such studies may have some taxonomic value, but are believed to be of greater value in the determination of possible phyletic and genetic relationships. The possibility of some correlation between the incubation period and retention of certain of the embryonic arteries as functionally modified vessels and particularly as ligamentous vestiges of the embryonic vessels has been indicated (Glenny, 1942 *b*; 1943). These embryonic vessels may remain as patent ducti, only partially occluded vestiges, or

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complete ligamentous vestiges, or the vessel may atrophy altogether and be entirely lacking.

Concerning the aortic arches and their derivatives, atrophy of the various portions of a vessel or of an entire arch occurs in an orderly series of fairly clear-cut steps. Of these, the left systemic (4th aortic) arch is the first (after the obliteration of the 1st and 2nd aortic arches) to atrophy completely. This occurs early in the embryonic life of the bird and is followed by an anastomosis of the left radix aortae (descending aorta) and the left pulmonary (6th aortic) arch medially to the ductus arteriosus or distal portion of the 6th aortic arch. The position of this anastomosis is marked (x) in Fig. 1. At the same time, the left ductus caroticus (dorsal portion of the radix aortae between the 3rd and 4th aortic arches) loses its posterior connection to the posterior ramus of the radix (due to the obliteration of the left 4th aortic arch) and becomes functionally modified through subsequent anastomoses with several small arteries which form *in situ* and come to supply the oesophagus, trachea, syrinx, and other tissues of the thoracic cavity, dorsal to the heart.

Shortly thereafter, the left ductus botalli (ductus arteriosus) begins to atrophy and some portions become completely obliterated either just before hatching or shortly after hatching (unpublished studies). At about the same time, the right ductus caroticus tends to lose its posterior connection (near the functional systemic arch) and becomes functionally modified in the same way as does the left ductus caroticus (Glenny, 1943). The new functionally modified vessels are then referred to as the ductus shawi (Glenny, 1940 *b*; 1943). In some cases, however, the ductus caroticus does not lose its embryonic connections entirely and may remain as a patent duct (Bhaduri, 1939; Finn, 1891; Glenny, 1940 *b*, 1943) or as the ligamentum caroticum (Finn, 1891; Glenny, 1940 *b*, 1942 *b*, 1943) as in certain doves, herons, ducks, the Sarus Crane, Hairy Woodpecker, and several of the Finches.

After this, and shortly before hatching, the left radix aortae and the dorsal portion of the right 6th aortic arch (ductus botalli) begin to atrophy (the extent of this atrophy is dependent upon factors not very clearly understood at the present writing, but probably of a genetic or phyletic character or both). Although the left radix remains as a white imperforate cord (ligamentum aortae) in most species of birds, it may nearly or even completely disappear in the surrounding fascia. Of the ultimate fate of the right ductus botalli, little more need be said except that it generally remains, either with a partial lumen (Glenny, 1941 *a*) or entirely occluded (ligamentum botalli). Further-

more, it may or may not atrophy completely at the proximal end. In many species of birds, however, the right ligamentum botalli fuses with the right radix aortae and may be observed only as a thin white line on the ventral or ventro-lateral face of the radix.

Anteriorly the internal carotid arteries carry blood to the head from the common carotid arteries. In most orders of birds, except some species of Psittaciformes, the internal carotids enter the hypapophysial

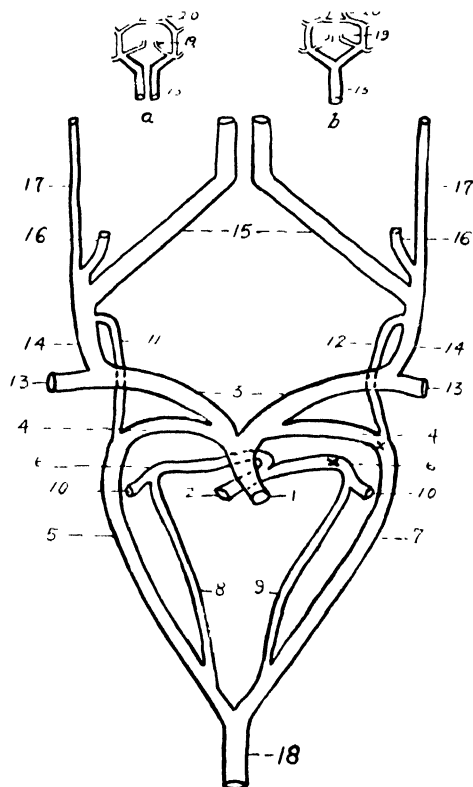


FIGURE 1

canal near the base of the neck and proceed anteriorly to about the 2nd or 3rd cervical vertebra, at which point they leave the canal and pass diagonally to the left and right sides of the neck, at the base of the lower jaw. The anterior portion of the external carotid then arises from the internal carotid trunk. According to Lillie, this is due to the fact that during early embryogeny, the internal and external carotids come to lie close to each other and with the subsequent elongation of the cervical

region, the anterior portions of the external carotids anastomose with the internal carotids which eventually come to lie in a ventro-medial position with reference to the cervical vertebrae. It is believed that, with the anastomosis of the internal and external carotids, the posterior portions of the external carotids finally come to serve as the lateral superficial cervical arteries of the adult bird.

In orders of birds which have but one internal carotid entering the hypapophysial canal so that the one alone carries the cephalic blood supply, as in the Piciformes (Glenny, 1943), Passeriformes (Glenny, 1942 *b*), some Casuariformes (Garrod, 1873; Glenny, 1942 *c*), Apteryx (Garrod, 1873; Glenny, 1942 *a*), Rhea (Garrod, 1873; Glenny, 1942 *c*), etc., there is, in addition to the internal-external anastomosis, a further anastomosis between the right and left internal carotids, at the anterior end (Fig. 1, *b*). In the Piciformes and again in the Passeriformes, the primary ascending-oesophageal artery appears to be the posterior portion of the right internal carotid artery (Glenny, 1943). Birds showing this condition are referred to by Garrod as "laevo-carotidinae," whereas species in which both left and right carotids enter the hypapophysial canal are said to be "bicarotidinae normales" (Beddard, 1898; Garrod, 1873; Glenny, 1940 *a*).

In the order Coraciiformes, both of the above arrangements have been observed by the writer and have likewise been noted by Garrod. Although one or the other is chiefly characteristic of a family, the Bucerotidae and Meropidae show the arrangements characteristic of both laevo-carotidinae and bicarotidinae normales in different species within each of the families. These will be considered further later on.

Garrod states that *Coracias garrula* and *Eurystomus* sp. of the Coraciidae; *Momotus lessoni*, Lesson, and *Eumomota superciliaris* (Sand.) Sharp, of the Momotidae; *Alcedo ispida*, *Halcyon* sp., *Dacelo gigantea*, *Dacelo cervina*, *Ceryle amazona* (Latham), *Ceryle maxima* (Pallas), and *Cittura cyanotis* of the Alcedinidae; *Buceros rhinoceros*, *Buceros plicatus*, *Buceros bicornis*, *Buceros coronatus*, and *Buceros atratus* of the Bucerotidae; and *Galbula albirostris* and *Urogalba paradisea* of the Galbulidae all present two carotid arteries, both of which enter the hypapophysial canal. He also points out that *Upupa epops*, Linné, *Merops apiaster*, Linné, and *Merops ornatus* of the Meropidae; *Trogon mexicanus* and *Trogon puella* of the Trogonidae; and *Toccos melanoleucus* (probably the same as *Lophoceros melanoleucos* (Licht.) of the Bucerotidae have but one vessel, the left internal carotid artery, entering the hypapophysial canal. Although Garrod included the Trogonidae in the Coccoyges, more recent taxono-

mists have further subdivided this order into the Coraciiformes, Coliiformes, Trogoniformes, Piciformes, and Cuculiformes.

The present writer treats these different orders in several papers (Glenny, 1941 *b*; 1943; and unpublished studies, AVES VI).

The materials used in this study were obtained from the Division of Birds, United States National Museum, and by the author.

MATERIALS³

CORACIIDAE:

Coracias caudatus (Linné), No. 290395⁴

Eurystomus orientalis (Linné), No. 290037

ALCEDINIDAE:

Ceryle rudis (Linné), No. 227104

Streptoceryle torquata (Linné) Ridgway, No. 18368 and No. 343960

Chloroceryle americana isthmica (Goldman) Ridgway, No. 343957

Chloroceryle amazona (Latham) Ridgway, No. 343956

Streptoceryle americana septentrionalis (Sharpe) Ridgway, No. 344807

Streptoceryle alcyon alcyon (Linné) Ridgway, No. 289745, and six other specimens

Alcedo atthis bengalensis, Gmelin, No. 289599

Alcedo coerulescens, Vieillot, No. 290194

Corythornis cristata, Sharpe

Dacelo novae-quineae (Hermann)

Halcyon chelicuti chelicuti (Stanley) Friedmann, No. 321639

Halcyon senegalensis (Linné), No. 18911

Halcyon (Sauropatis) chloris (Boddaert), No. 318068

Halcyon cinnamominus, Swainson, No. 318051

BUCEROTIDAE:

Rhytidoceros (Rhyticeros) undulatus (Shaw), No. 20229

Lophoceros melanoleucos (Licht.) *alboterminatus*, No. 19012

Lophoceros melanoleucos (Licht.) subsp., No. 22087 and No. 321513

Lophoceros flavirostris (Rüppell), No. 289859

MEROPIDAE:

Melittophagus variegatus (Vieillot), No. 226592

Merops apiaster, Linné, No. 291227

Merops superciliosus, Linné, No. 291228

Aerops albicollis albicollis (Vieillot), No. 18926

³Cory (1918) gives the following names as correct for five species of American Kingfishers: Great-ringed Kingfisher, *Streptoceryle t. torquata* (L.); Belted Kingfisher, *streptoceryle a. alcyon* (L.); Amazon Kingfisher, *Chloroceryle amazona* (Latham); Isthmian Green Kingfisher, *Chloroceryle americana isthmica* (Goldman); and the Texan Green Kingfisher, *Chloroceryle americana septentrionalis* (Sharpe).

⁴Numbers following the names refer to specimens from the United States National Museum collection of alcoholics.

Merops orientalis (Latham), No. 291480

Merops pusillus pusillus (Muller), No. 18928

TODIDAE:

Todus multicolor, Gould, No. 225868

Todus angustirostris, Lafresnaye, No. 291047

Todus subulatus, Gray, No. 289645

Todus hypochondriacus, Bryant, No. 223955

Todus mexicanus, Lesson, No. 226997

MOMOTIDAE

Urospathi martii martii (Spix) Ridgway, No. 321524 and No. 18725

Eumomota superciliosa superciliosa (Sandbach) Ridgway, No. 81633

Momotus caeruleiceps (Gould) Ridgway, No. 61477

Momotus subrufescens conexus (Thayer and Bangs) Ridgway, No. 343962

UPUPIDAE:

Upupa epops epops (Linné), No. 291229

Upupa africana, Bechstein, No. 227155

PHOENICULIDAE:

Phoeniculus purpureus, Sclater, No. 321529^a

Routine dissections were made on each of the above species and diagrams of the arrangement of the arteries in the neck and thorax prepared. The results are set forth in the following observations which are based on the dissection of single specimens, except in the case of *Streptoceryle t. torquata*, *Streptoceryle a. alcyon*, *Lophoceros melanoleucos*, and *Urospathi m. martii* as noted above.

OBSERVATIONS

Species of the Family Alcedinidae in this study are bicarotidinae normales and further present the following generalized arterial arrangement in the region of the neck and thorax (Figs. 2 and 3). The left and right innominates (2) arise from the aortic root (1). The functional systemic (right 4th aortic) arch (3) branches from the right innominate and connects with the right descending aorta (*radix aortae*) (4) to join the dorsal aorta (5). The ligamentous vestige of the left *radix aortae* (*ligamentum aortae*) (6) persists in the adult except in a few species where it either is functionally modified (Glenny, 1939; 1940 a) or presents a lumen for part of its length. In general, the

^aThis species was formerly placed on the Family UPUPIDAE and represents the form described as *Upupa erythrorynchus*, Latham. This revision was made by Sclater (1924) in his *Systema Avium Ethiopicarum*, which was published by the British Ornithological Union.

right ligamentum botalli (7) remains as a vestige of the embryonic ductus botalli or ductus arteriosus.

The subclavian artery (9) sends off the coracoid major (10), axillary (11), intercostal (12), and two pectoral (13) arteries. The common carotid (26) gives rise to the ductus shawi (16), superficial cervical (20 and 21), vertebral (23), and internal carotid (27) arteries.

The coracoid minor (14) arises from the axillary artery shortly after its origin from the subclavian artery. The coracoid major artery gives rise to a short sterno-tracheal artery (15) which supplies the sterno-tracheal muscle.

The right superficial cervical artery gives rise to a basi-cervical (24) artery and a subscapular (22) artery before proceeding anteriorly along the neck, where it sends off small branches to the cervical lymph glands, connective tissues, and musculature of the neck. Arising separately near the base of the vertebral artery is the primary ascending-oesophageal artery. The left superficial cervical and basi-cervical arteries arise near the base of the left vertebral artery.

The intercostal artery (12) arises from the subclavian artery between the axillary and pectoral arteries. A short meso-oesophageal artery arises from the left ductus shawi. Both left and right internal carotids enter the hypapophysial canal and proceed anteriorly to the head without anastomosing. Both the right ligamentum botalli (7) and the left ligamentum aortae (6) are present.

The above condition is found in *Alcedo coerulescens* and *Alcedo atthis bengalensis*. Slight variations from this are found in other species and genera and are noted below.

Corythornis cristata and *Dacelo novae-guineae* are like *Alcedo* (above) except that the right ligamentum botalli becomes nearly or completely obliterated. The left ligamentum aortae is very prominent. The right ligamentum botalli and left ligamentum aortae are more prominent in *Chloroceryle amazona* than in *Alcedo*.

Chloroceryle americana isthmica and *Streptoceryle alcyon* differ from *Chloroceryle amazona* in that the left radix aortae appears to be functionally modified and serves as a dorsal intercostal artery sending off minute branches along its course anteriorly.

The left ligamentum aortae is very prominent, presenting a very short lumen, in *Ceryle rudis*. The right ligamentum botalli is likewise prominent. The intercostal artery does not arise near the base of the pectoral arteries but originates from the subclavian artery near the coracoid major artery.

Members of the genus *Halcyon* vary somewhat in detail in that the

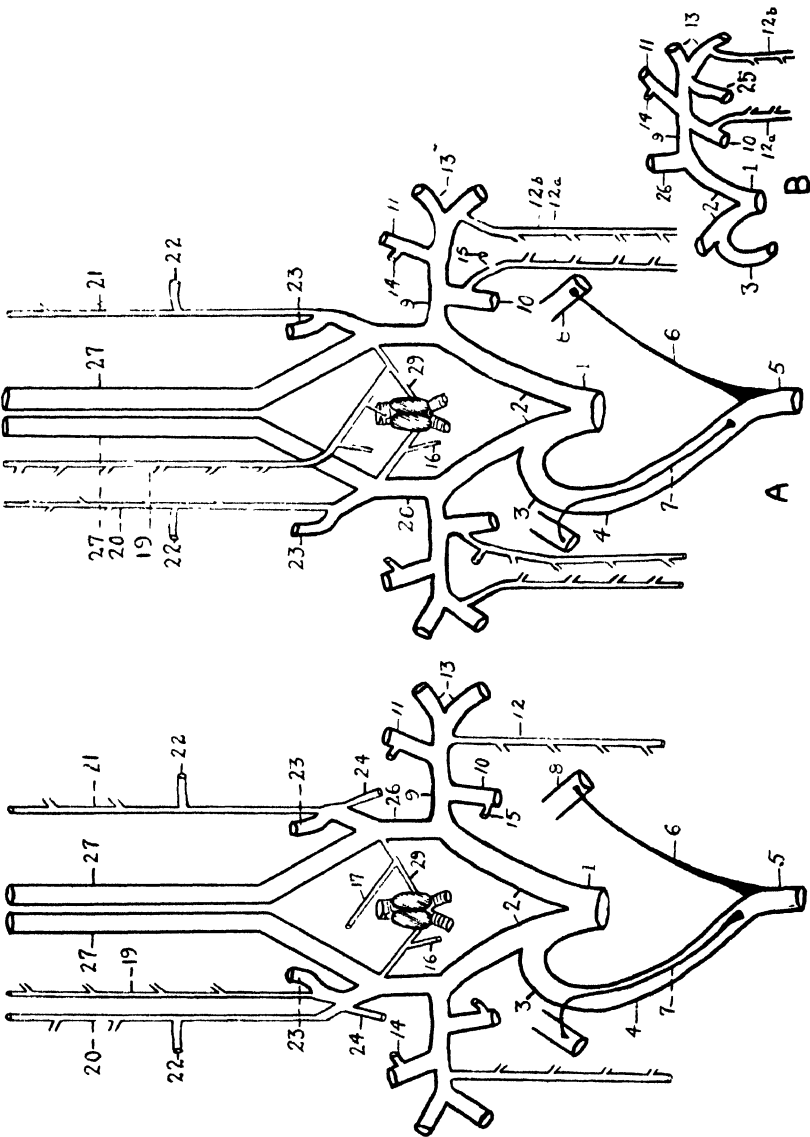


FIGURE 3

FIGURE 2

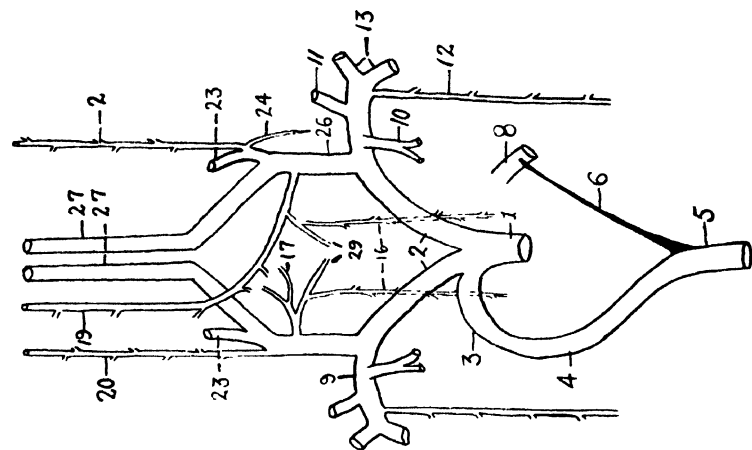


FIGURE 6

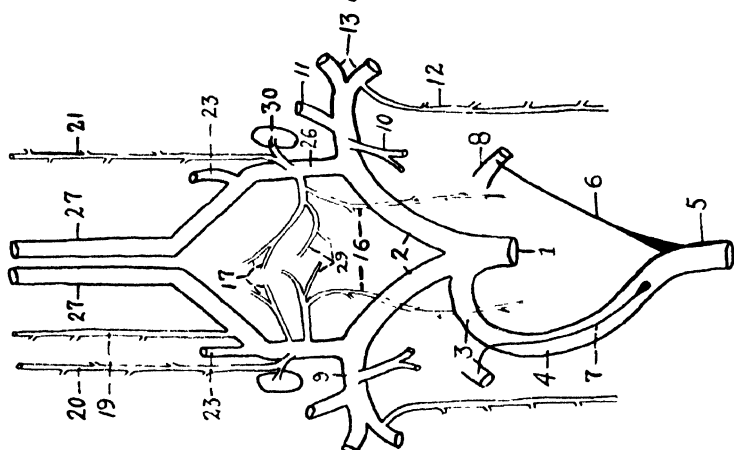


FIGURE 5

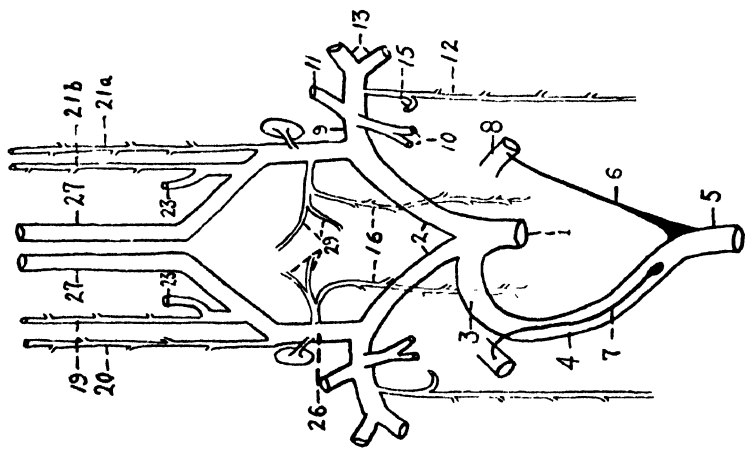


FIGURE 4

primary ascending-oesophageal artery arises from the left ductus shawi (probably due to *in situ* anastomosis during embryonic development) instead of from the right vertebral artery as in *Alcedo*. Furthermore, the ventral intercostal artery (12a) arises from the coracoid major while the lateral intercostal artery (12b) arises from the posterior pectoral artery (Fig. 3B).

In *Halcyon chelicuti*, the right ligamentum botalli is much reduced and becomes almost entirely obliterated through fusion with the right radix aortae. The ventral intercostal arteries are placed more toward the base of the coracoid major and may arise separately from the subclavian arteries.

The right ligamentum botalli is greatly reduced and almost entirely obliterated through fusion with the right radix in *Halcyon (Sauropatis) chloris*, while the left radix retains a lumen for a considerable distance.

Halcyon cinnamomimus may or may not retain a short, minute lumen in the left ligamentum aortae and, in much older specimens, the lumen probably disappears entirely.

In the single specimen of *Halcyon senegalensis* which was included in this study, two coracoid major arteries were observed. The one the normal (coracoid major prima) (10) and a second (coracoid major secunda) (25) (Fig. 3B) vessel which arises from the subclavian artery between the axillary and pectoral arteries. The right ligamentum botalli is much reduced and tends to fuse with the right radix aortae.

The entire arrangement-pattern of arteries in the neck and thorax of *Streptoceryle torquata* more closely resembles that of *Halcyon* than of the group which includes *Alcedo* and the species of *Chloroceryle* and *Streptoceryle* except that there is but one intercostal artery present in *S. torquata* while *Halcyon* presents two as already mentioned above. In this respect it follows the general plan or pattern of *Alcedo*. The intercostal blood supply is derived from the intercostal artery which arises from the posterior pectoral artery or from the subclavian artery at the base of the pectoral arteries. The coracoid major secunda which is present in *Halcyon* is lacking in *Streptoceryle torquata*. The ligamentum aortae is present, while the right ligamentum botalli fuses readily with the right radix aortae.

Somewhat intermediate between the arrangement-pattern of *Halcyon* and the type observed in the other species already mentioned above is that found in *Streptoceryle americana septentrionalis*. The arrangement-pattern in this case is chiefly like that illustrated in Fig 3A with certain minor differences.

The primary ascending-oesophageal artery arises from the left ductus

shawii as in *Halcyon*. The intercostal arteries arise separately from the subclavian arteries between the axillary and pectoral arteries as in Alcedo *et al.* The left radix aortae remains as a functionally modified dorsal intercostal artery, as is the case in *Streptoceryle alcyon* and *Streptoceryle americana isthmica*, while the right ductus botalli remains as a prominent ligamentous vestige. In the one specimen dissected for this study, the right superficial cervical arises separately from the common carotid artery and not from the vertebral trunk near its base, while the right subscapular artery arises from the vertebral artery shortly after its origin from the right internal carotid artery. In other respects, the arrangement is like that for the Family Alcedinidae.

The arrangement-pattern of main arteries in the neck and thorax of the two species of Coraciidae which were studied is essentially like that of Alcedo, but differs in a few points (Fig. 4).

The lateral superficial cervical arteries (20 and 21a) arise from the common carotids before they bifurcate to form the vertebral (23) and internal carotid (27) arteries. The right vertebral gives rise to the primary ascending-oesophageal artery (19) while the left vertebral may or may not give rise to a more ventral superficial cervical artery (21b). The intercostal arteries (12) arise from the subclavians (9) between the axillary (11) and pectoral (13) arteries and send off a small sterno-tracheal artery (15) almost at once, before sending off branches to supply the intercostal muscles.

In *Coracias caudatus* the right ventral superficial cervical artery is present, whereas in *Eurystomus orientalis* it appears to be lacking. The ligamentum aortae (6) is very prominent in both species, while the ligamentum botalli (7) is prominent in *Coracias* and almost entirely lacking, except for the presence of a basal distal butt, in *Eurystomus*.

Of the Todidae thus far studied (Fig. 6), the arrangement-pattern is that of bicarotidinae normales. The primary ascending-oesophageal artery (19) is a branch of the left ductus shawii (16); the left superficial cervical (21) arises as a branch from the vertebral artery (23) near its base along with the unilateral basicervical artery (24); the right superficial cervical artery (20) arises from the common carotid artery (26), just posterior to the right vertebral artery, and serves as an accessory ascending-oesophageal artery in addition to serving as the main supply for the cervical lymph glands, connective tissues, and part of the cervical musculature.

The subclavian artery (9) sends off the coracoid major (10), the axillary (11), the intercostal (12), and two pectoral (13) arteries in turn. The ligamentum aortae (6) is present in each of the five species

studied, while the ligamentum botalli is almost or completely atrophied and may or may not fuse in part or entirely with the radix aortae (4). It could be observed with difficulty in *Todus subulatus* and *Todus mexicanus*.

In the single specimen of *Todus angustirostris*, the right internal carotid artery appears to be wanting so that the left internal carotid alone enters the hypapophysial canal. This may or may not be a specific characteristic; it is, however, a singular and interesting case since the writer could not detect its presence either at the distal end of the right common carotid artery or at any place in the hypapophysial canal in the basal region of the neck.

The basic arrangement-pattern of the arteries in the neck and thorax of the Momotidae differs from that found in the other families of Coraciiformes (Fig. 5).

Left (21) and right (20) superficial cervical arteries arise from the common carotids (26) and are not so closely associated with the vertebral arteries (23) as is the case in the species already described. Their attachment to the common carotids is formed at the site of the thyroid gland (30) and near the origin of the ductus shawi (16). The primary ascending oesophageal artery (19) is a separate vessel in *Momotus caeruleiceps* and *Urospathi martii*, while in *Momotus s. conexus* and *Eumomota superciliosa* the right superficial cervical (20) also serves as an ascending-oesophageal artery. The intercostal arteries (12) arise from the subclavian artery (9) at a point between the coracoid major (10) and axillary (11) arteries in *Urospathi martii*; from the subclavian artery between the axillary (11) and pectoral (13) arteries in *Momotus s. conexus*; and from the posterior pectoral (13) artery in *Momotus caeruleiceps* and *Eumomota superciliosa*.

The ligamentum aortae (6) is prominent in each of these species and presents a short lumen in *Urospathi* and both species of *Momotus*. The right ligamentum botalli (7) is fused with the right radix (4) and remains as a fine, white streak on the ventral face of the radix. It is very prominent in *Momotus s. conexus* and slightly less prominent in *Momotus caeruleiceps*.

In the Bucerotidae (Figs. 7 and 8), both left and right internal carotids (27) may enter the hypapophysial canal or the right internal carotid may be abortive or degenerate (28) during embryogeny so that it serves the ventral cervical musculature at the posterior end of the canal. The left internal carotid then alone enters the hypapophysial canal in these species. Garrod has already indicated that certain species of the Bucerotidae are "bicarotidinae normales" while others are "laevo-

carotidinae" as is the case in *Toccus* (*Lophoceros*) *melanoleucus* (Garrod, 1873). The writer feels that the three species which presented this abortive or degenerate right internal carotid artery deserve further and more extensive careful investigations to determine whether or not they represent a distinctive line of development, and to determine if possible at what point the right internal carotid artery undergoes this radical functional modification (as embryological evidences).

The basic arrangement-pattern of the main arteries in the neck and thorax, aside from the singular condition of the internal carotid arteries is essentially the same in each of the species studied. The innominate arteries (2) divide to form the subclavian (9) and common carotid (26) arteries. The subclavian arteries then give rise to the coracoid major (10), axillary (11), intercostal (12), and two pectoral (13) arteries in order. The common carotids give rise to the thyroid artery (30), ductus shawi (16), superficial cervical (20 and 21), ascending-oesophageal (19), and vertebral (23) arteries before becoming the internal carotid (27) arteries which, with the already mentioned exception, enter the hypapophysial canal and pass forward to supply the cephalic region.

The right superficial cervical (20) supplies the cervical lymph glands and some of the cervical musculature as well as serving as an accessory ascending-oesophageal artery. The left superficial cervical (18) sends off the primary ascending-oesophageal (19) artery and a lateral superficial cervical artery (21) which supplies the cervical lymph glands and accessory tissues of the neck. In *Rhytidoceros undulatus*, two lateral superficial cervicals were observed to be present.

The subscapular arteries (22) join the vertebral arteries (23) near their base. The axillary (11) arteries give rise to small coracoid minor (14) arteries. The sterno-tracheal artery (15) joins the coracoid major (10) in *Rhytidoceros undulatus*, whereas it joins the intercostal artery (12) in *Lophoceros melanoleucos subspp.* and *Lophoceros flavirostris*.

Of the four species studied, *Rhytidoceros undulatus* presented both left and right internal carotids of equal size, and both entered the hypapophysial canal to pass to the head, whereas in the three species of *Lophoceros* the left internal carotid alone entered the canal while the presumed abortive or functionally modified right internal carotid (28) ends at the base of the canal and serves to supply a few of the cervical muscles in that area. It is greatly reduced in diameter and appears as a very short ductus. In these three species of *Lophoceros*, the left internal carotid bifurcates into dextro- and laevo-cephalic carotids (Fig.

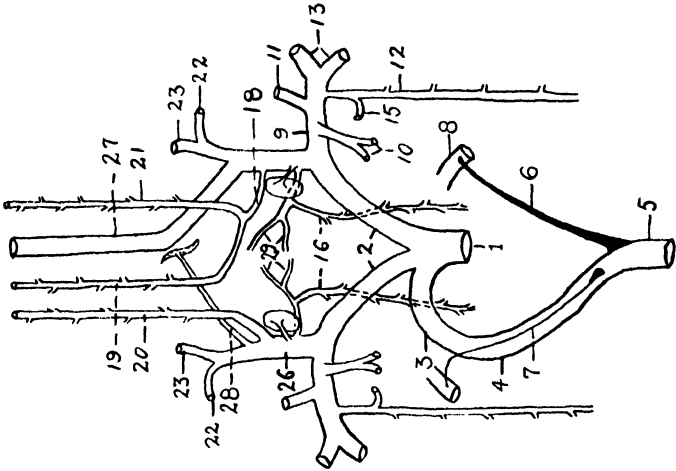


FIGURE 8

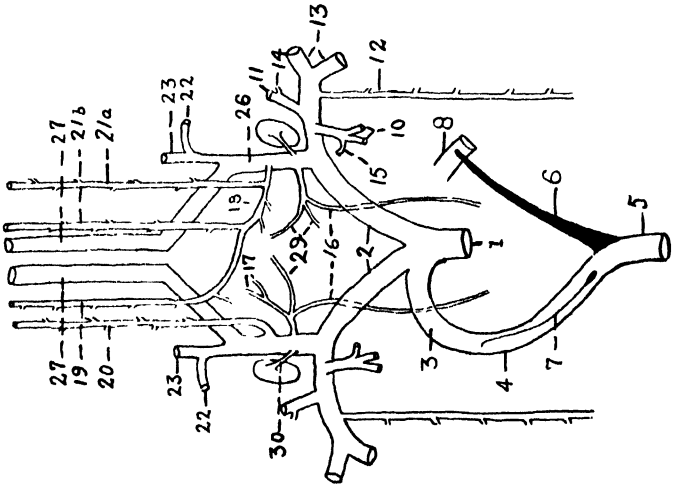


FIGURE 7

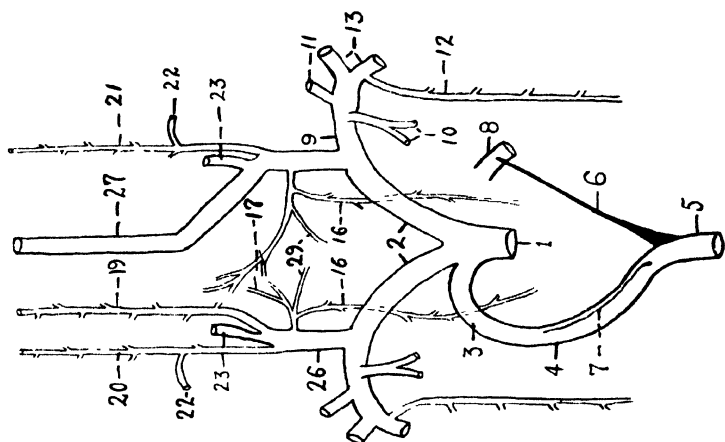


FIGURE 11

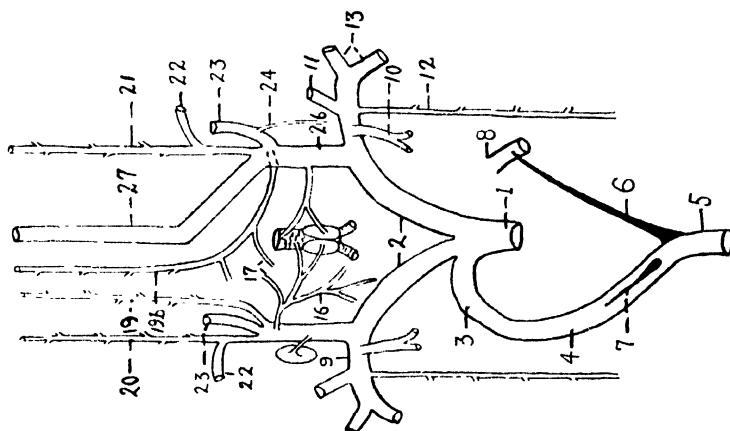


FIGURE 10

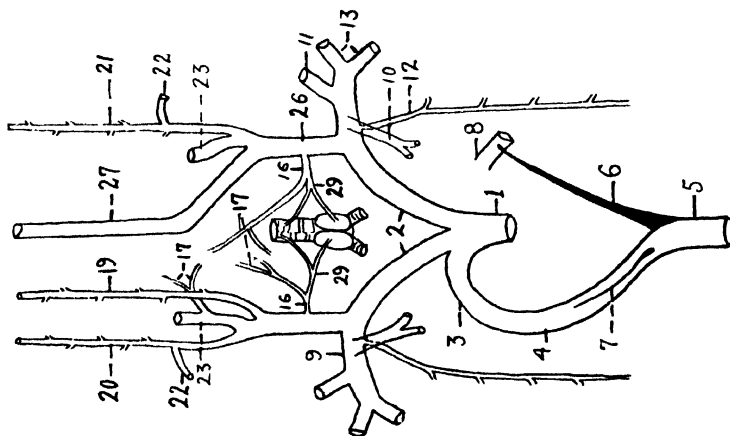


FIGURE 9

1b). These later divide into their component cephalic internal and external carotid branches.

The ligamentum aortae (6) is present in each of the four species, as is the right ligamentum botalli (7). The latter varies in the degree of atrophy, however, and is largest in *Lophoceros flavirostris*, reduced in *Lophoceros melanoleucos* subpp., and short with a tendency to fuse with the radix in *Rhytidoceros undulatus*.

In the Meropidae (Fig. 11), as in other birds, the aortic root (1) gives rise to the left and right innominate (2) arteries. The right systemic arch (3) arises from the right innominate artery at or near the base shortly after the division of the aortic root. The innominates divide to form the common carotid and subclavian arteries. The subclavian arteries give off the coracoid major, axillary, and two pectoral arteries in order; while the common carotids give off the ductus shawi, superficial cervical, vertebral, and internal carotid arteries. The subscapular arteries are attached to the lateral superficial cervical arteries. In *Mcrops orientalis*, *Merops apiaster*, *Merops superciliosa*, and *Aerops a. albicollis* the left internal carotid alone enters the hypapophysial canal; while in *Mcrops p. pusillus* and *Melittophagus variegatus* both left and right internal carotids enter the canal.

The primary ascending-oesophageal artery (19) arises from the right common carotid in each of the species studied. A short meso-oesophageal artery (17) is received as a branch from the left ductus shawi in *Merops orientalis*, *Merops apiaster*, and *Melittophagus variegatus*; while an accessory ascending-oesophageal artery is received from the left ductus shawi in *Aerops a. albicollis* and *Mcrops p. pusillus*.

The intercostal arteries arise from the posterior pectoral artery near its origin from the subclavian artery. The ligamentum aortae is present in all species, while the right ligamentum botalli appears to be wanting in each of the specimens which was studied.

The arterial arrangement in *Phoeniculus purpureus* (Fig. 10) is quite similar to that of *Aerops albicollis*, but varies in that the intercostal artery lies opposite the axillary artery; a short basi-cervical artery (24) arises from the left vertebral artery near its base; and the accessory ascending-oesophageal artery (19b) does not join the left ductus shawi, but arises from the left common carotid artery near the base of the vertebral artery. The ligamentum aortae is present, as a short portion of the ligamentum botalli which tends to fuse to the right radix aortae.

The arterial arrangement in both *Upupa epops epops* and *Upupa africana* is the same (Fig. 9). The intercostal arteries arise from the posterior face of the subclavian arteries near the base of the coracoid

majors. A meso-oesophageal artery (17) arises from the left ductus shawi in both species and another from the right vertebral artery in *Upupa epops epops*. The left internal carotid alone enters the hypapophysial canal, while its complimentary vessel of the right side serves as the primary ascending-oesophageal artery. The ligamentum aortae is present and prominent in both species, while the right ligamentum botalli is present and tends to fuse with the right radix aortae.

DISCUSSION

It will be seen from the above observations on the arteries of the neck and thorax of the Coraciiformes, that there is considerable variation in arrangement-patterns: first, between the different families within the order and second, within any one of the families. This is particularly noticeable in the Bucerotidae and in the Meropidae with reference to the presence of both left and right internal carotids within the hypapophysial canal, or the absence of functional modification of the right vessel.

The present observations on the two subspecies of *Lophoceros melanoleucus* confirms to an extent the previous observations of Garrod on *Toccus melanoleucus*.

Irregularity in constancy of presence of both internal carotid arteries within the hypapophysial canal might lead one to suspect that the order Coraciiformes could be further subdivided, or that there might be a tendency toward the formation of two or more widely divergent groups of birds.

It is interesting to note that under older systems of classification *Phoeniculus purpureus* was placed among the Upupidae. Certain fairly close relationship is to be noted in the arterial arrangement-patterns of these two families of birds.

The present writer feels that embryological studies on the various stages of development of the various families and species of birds within this group would probably explain the manner in which the right internal carotid artery becomes shifted from the expected position to the final adult position. Such studies should prove to be of great value in aiding the phylo-geneticist in his studies and at the same time they should help immeasurably in the work of the taxonomist.

ACKNOWLEDGEMENTS

The writer wishes to acknowledge his gratitude to Dr. Alexander Wetmore, Assistant Secretary, Smithsonian Institution, and Dr. Herbert Friedmann, Curator, Division of Birds, United States National Museum,

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CAPTIONS FOR FIGURES

FIGURE 1

Diagrammatic representation of the Aortic Arches and Associated Main Arteries in the Neck and Thorax of Birds—(to illustrate the series of changes which take place during middle-late embryogeny). Ventral view.

(Two small diagrams at top of page)—Cephalic branching of internal and external carotid arteries: *a.* bicarotidinae normales, *b.* laevo-carotidinae.

KEY TO ABBREVIATIONS

1, aortic root; 2, pulmonary root; 3, innominate artery; 4, systemic (4th aortic) arch; 5, right radix aortae (aorta descendens); 6, pulmonary (6th aortic) arch; 7, left radix aortae; 8, right ductus botalli; 9, left ductus botalli; 10, pulmonary arteries; 11, right ductus caroticus; 12, left ductus caroticus; 13, arteria subclavia secunda; 14, ventral aorta; 15, internal carotid artery (adult position); 16, vertebral arteries; 17, definitive or adult superficial cervical arteries; 18, dorsal aorta; 19, external carotid (maxillary) artery; 20, internal carotid artery; \times , marks the approximate position of the anastomosis between the left radix aortae and the left pulmonary arch (proximal portion).

FIGURES 2-11

VENTRAL VIEW OF MAIN ARTERIES IN THE NECK AND THORAX OF

FIGURE 2.—Alcedo.

FIGURE 3.—Halcyon.

FIGURE 4.—Coracias caudatus.

FIGURE 5.—Momotus caeruliceps.

FIGURE 6.—Todus multicolor.

FIGURE 7.—Rhytidoceros undulatus.

FIGURE 8.—Lophoceros melanoleucos
alboterminatus.

FIGURE 9.—Upupa epops epops.

FIGURE 10.—Phoeniculus purpureus.

FIGURE 11.—Merops orientalis.

KEY TO ABBREVIATIONS

1, aortic root; 2, innominate artery; 3, systemic (4th aortic) arch; 4, radix aortae; 5, dorsal aorta; 6, ligamentum aortae; 7, ligamentum botalli; 8, pulmonary artery; 9, subclavian artery; 10, coracoid major (prima) artery; 11, axillary artery; 12, intercostal artery; 13, pectoral arteries; 14, coracoid minor artery; 15, sterno-tracheal artery; 16, ductus shawi; 17, meso-oesophageal artery; 18, cervico-oesophageal artery; 19, ascending-oesophageal artery; 20, right superficial cervical artery; 21, left superficial cervical artery; 22, subscapular artery; 23, vertebral artery; 24, basi-cervical artery; 25, coracoid major (secunda) artery; 26, common carotid artery; 27, internal carotid artery; 28, abortive or functionally modified right internal carotid artery; 29, syrinx and tracheal arteries; 30, thyroid artery and thyroid gland.

THE PILCHARD *SARDINOPS CAERULEA* (Girard) ON
CANADIAN FISHING GROUNDS WITH SPECIAL
REFERENCE TO AN UNUSUAL ABUNDANCE
OF YOUNG FISH

By JOHN LAWSON HART, F.R.S.C.

THIS report presents a contrast between the usual behaviour of pilchards in relation to the Canadian fishery and the unusual features in the occurrence in Canadian Pacific waters of an abundant group of young pilchards.

USUAL BEHAVIOUR OF PILCHARDS

Time of occurrence of pilchards in Canadian waters

Typically the pilchard fishery in Canada is one of the summer and autumn. The main bodies of pilchards usually appear off the Canadian coast between the middle of June and the end of July and remain until some time between the middle of September and the middle of October.

Various exceptions have occurred to these usual conditions. In the eighteen years since the introduction of the pilchard reduction industry in 1925, there have been three years, 1933, 1937, and 1939, in which no substantial schools of pilchards were observed in waters off the Canadian coast.

In 1925, 1938, and 1941, regular fishing continued later into the fall than in other seasons, and following the last-named season substantial catches were made during the winter of 1941-2 both in the inlets and outside on the west coast of Vancouver Island.

In many years a few pilchards have remained in the inlets during the winter, and sometimes these have yielded a few hundred tons of fish when encountered by herring fishermen during the winter months.

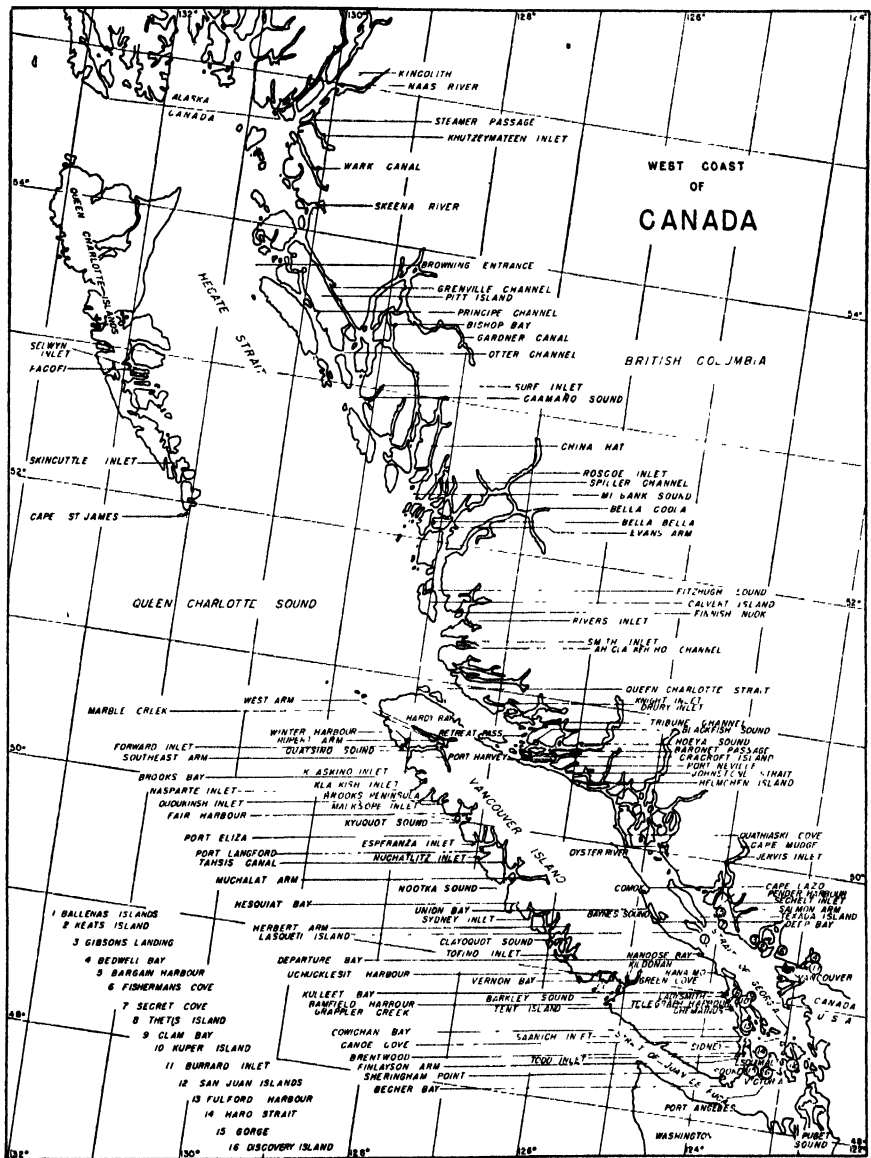
Occasional pilchards are found in herring catches during the winter months both on the west coast of Vancouver Island and in the Strait of Georgia. As a rule, pilchards have been sparsely distributed in the catches, and only in catches made during the winter of 1941-2 did pilchards comprise a significant part of the herring catches. During that season only was any confusion possible as to whether herring were present in pilchard schools or pilchards were present in herring schools. (One such mixed catch was reported in the Strait of Georgia in 1942-3.)

Places where pilchards are generally observed

The typical northern limit of pilchard occurrence is the west coast of Vancouver Island. As mentioned above, there have been three seasons in recent years in which pilchards were not found in that area in spite of thorough scouting of the waters out to fifty miles off-shore. In each of these years pilchards were taken off the coasts of Washington and Oregon. That the usual Canadian population was present on the fishing grounds off the coast of the north-west United States is not a necessary conclusion since in several years, 1935, 1936, 1937, 1938, and 1941, successful fisheries were conducted off the Canadian coast as well as off the coasts of Washington and Oregon.

In most seasons fish have been generally distributed among the larger inlets and sounds of the west coast of Vancouver Island or generally distributed off-shore. Presumably, pilchards were more regularly available in the central and south-eastern part of the west coast since none of the seven reduction plants built in Kyuquot and Quatsino sounds have continued to operate to the present time. Other factors such as distance from Vancouver may have entered into the failure of these plants to continue in operation but the observation confirms the statement of a responsible representative of the industry that the supply of pilchards north of Esperanza Inlet was too uncertain to support a profitable industry. Although fishing is on the whole more steady off the central and southern parts of the west coast of Vancouver Island, there have been two seasons in which fishing was limited during the later part of the season to the north-western part of Vancouver Island—1938 and 1941. In each of these years the fish entered the inlets and provided very good fishing for two months or more. There does not appear to have been a year in which pilchards have remained for any extended time in Barkley Sound or Clayoquot Sound to the complete exclusion of the rest of the west coast area.

From 1917 to 1924, when the pilchard fishery was operated only to supply fish for canning, and during the first two or three years of the reduction industry which began in 1925, fishing for pilchards was rather well confined to the inlets of the west coast of Vancouver Island. From this condition, the fishery changed progressively to one pursued outside the inlets. In the third year of the more intense fishery, it became necessary for seine boats to venture into the open Pacific to maintain regular catches and by 1929 most of the pilchards taken were caught in exposed waters. It is probable that in every season thereafter some fish were taken in the inlets, but for a number of years outside fish comprised



more than 80 per cent of the total catch. The trend away from inlet fishing, if such existed, has been broken or reversed by the successful exploitation of abundant schools of pilchards in the inlets in the north-western part of Vancouver Island in 1938, in 1940, and to a less extent in 1941.

Although the west coast of Vancouver Island is the usual northward limit of pilchard migration, they have, during several seasons, been recorded north of there in significant quantities. In 1931, pilchards penetrated considerably farther north than had been previously recorded and it is probable that they were sufficiently abundant to support a substantial commercial fishery although, owing to lack of facilities for handling the catch, the take was limited to 98 tons caught near Rivers Inlet for bait. In that season pilchards were recorded (Schultz, Hart, and Gunderson, 1932) from Tribune Channel and Knight Inlet off Queen Charlotte Strait; Smith Inlet and Gardner Canal in central British Columbia; Cape St. James and Skincuttle Inlet on the Queen Charlotte Islands; and in northern British Columbia near the Skeena River, and at several places in the Naas Estuary including, at Kincolith, near Wark Canal, and in Steamer Passage. In August of the same year, a number of pilchards were taken from herring catches made near Cape Ommaney in Alaska and were recorded from Pender Harbour and Quathiaski Cove off the Strait of Georgia. In 1932, 129 tons of pilchards were recorded as being taken for bait in central British Columbia but there were no widespread reports of general abundance. In 1936, commercial catches amounting to 5,563 tons were reported, principally from the inlets between Bella Bella and Bella Coola. The circumstances surrounding the development of considerable fisheries in central British Columbia in 1941 and 1942 will be dealt with later.

Lengths of pilchards usually observed

The lengths of pilchards prevalent in the British Columbia fishery from 1929 to 1939 are indicated in Table I. For each year are given for males and females the average standard lengths in millimetres and the limits of the length range which include the median 80 per cent of the individuals as indicated by an extensive sampling programme. Averages marked with an asterisk were obtained by averaging arithmetic means from two sampling stations.

These values are in keeping with those obtained by less thorough samplings carried out in 1926 and 1927.

TABLE I
REPRESENTATIVE LENGTHS OF PILCHARDS SAMPLED FROM THE COMMERCIAL CATCH

Year	MALES			FEMALES		
	Lower decile	Average	Upper decile	Lower decile	Average	Upper decile
1929	237	247.7	259	242	252.8	264
1930	237	246.8*	257	242	252.4*	263
1931	229	241.6*	253	233	246.8*	260
1932	233	244.0*	257	235	248.2*	262
1933	234	245.5	257	237	249.7	264
1934	238	249.5	258	243	252.9	264
1935	239	250.0	261	244	254.8	266
1936
1937	235	248.0	261	239	252.9	267
1938	234	246.4	259	238	251.0	264
1939	227	241.0	253	230	245.4	260

TABLE II

Year	MALES			FEMALES		
	Lower decile	Average	Upper decile	Lower decile	Average	Upper decile
1926	226	243.4	262	233	251.8	271
1927	231	244.8	259	236	249.4	265

Evidently pilchards have been of fairly constant size from year to year and their lengths are rather closely grouped around the average. As more than 85 per cent of pilchards are sexually mature at a length of 200 mm., it is evident that maturity is not the main stimulus controlling migration into the sphere of the Canadian fishery, and, consequently, typical pilchards taken by Canadian fishermen may be described as large mature fish. A few small fish are encountered occasionally in the sampling of the commercial run, and in three of the twelve years tabulated (1931, 1932, and 1933) five or more pilchards less than 200 mm. in standard length were encountered in the samples, but they were never present in sufficient numbers to affect materially the position of the lower decile.

Schools of small fish have occasionally been encountered in Canadian waters. A few small pilchards with standard lengths around 155 mm. were taken in the winter of 1930-1 in Barkley Sound. The fisheries inspector in the Kyuquot area reports, "In the year 1932 large schools of immature pilchards were observed in these waters . . ." In the same year the fisheries officer for Nootka reported, "fairly large bodies frequented the inside waters from early May to June 26." Again in the winter of 1932-3 small pilchards about 150 mm. in standard length

were present in Barkley and Nootka Sounds, and during the following summer a few schools consisting mainly of small pilchards between 170 and 180 mm. standard length were taken in Barkley Sound by small-meshed seines. Young pilchards presumably in their first year were reported in small numbers among the herring during the winter of 1940-1.

The year 1936 differed from all previous seasons in the presence among the northern pilchards on the fishing grounds of an abundant group of small fish. In samples, the small fish constituted nearly 25 per cent of all the fish taken but their relative abundance on the fishing grounds was certainly higher since there was a conscious effort on the part of fishermen to avoid the small fish which fouled their nets and were unproductive of oil. There was a marked tendency for large and small fish to be segregated although a few catches were sampled in which large and small pilchards were about equally represented. Considering the groups above and below 213 mm. standard length separately gave the following sets of characteristic values :

TABLE III

Standard length	MALES			FEMALES		
	Lower decile	Average	Upper decile	Lower decile	Average	Upper decile
Less than 213 mm.	186	194.1	202	187	195.3	205
More than 213 mm.	230	243.6	257	232	248.0	262

The group of small pilchards appeared for one season only: survivors were not encountered in significant numbers during the 1937 season or in subsequent seasons in which they would have been distinguishable.

Mortality among pilchards in Canadian waters prior to 1940

Dead marine organisms are usually returned so quickly to the vital cycle that mortality must be very high or special circumstances must exist if epizootics are to be observed. This is especially true in the summer months when warmer water accelerates the general rhythm of growth and decay. This may be the reason that no epizootics have been recorded among pilchards during the summer months when they are most abundant in British Columbia waters. On the other hand, the absence of records may indicate that excessive mortalities have not taken place.

Small groups of pilchards which remain at the heads of the inlets at various places in British Columbia during the winter are reported

to have experienced mortalities on a number of occasions at such places as Ah cla ker ho Channel off Smith Sound, Seymour Inlet, Drury Inlet, Tahsis Canal, Deserted Creek, Muchalat Arm, Herbert Arm, Tofino Inlet, Bamfield Harbour or Grappler Creek. These have been ascribed either to excessive amounts of fresh water or to low temperatures, one of which conditions is almost always present at the heads of the inlets during the winter months. It is reasonable to suppose that most epizootics in the heads of inlets would be observed although it is unlikely that all of them would be reported.

1939 YEAR-CLASS IN CANADIAN WATERS

In the foregoing paragraphs the behaviour of pilchards in Canadian waters has been described along with an outline of the variations which can be expected to occur and which may be classified as usual. The group of small pilchards which appeared on the British Columbia coast in 1940, however, differed so markedly from the small fish present in other years in both qualitative and quantitative aspects of their behaviour that they have been made the subject of special study.

Time of first occurrence

The first record of these young pilchards was received on May 11, 1940 in a telegram stating that immature pilchards 3 or 4 inches long were present in Barkley Sound and especially in Uchucklesit Harbour. It is likely that the fish had been present and observed for approximately a week before the telegram was sent, but it is improbable that they were present for much longer, since the fisheries officer's report states that pilchards were in the area "from early May." Later investigation showed the identification of the species to be correct.

During early summer immature pilchards were recorded from numerous points on the west coast of Vancouver Island. The records of first appearance as submitted by fisheries officers follow: Clayoquot Sound area (Sydney Inlet and Herbert Arm), latter part of May; Nootka Sound area, middle of May; Kyuquot Sound area (Nasparte, Ououkinsh, and Malksope Inlets), May 28 and 29, and in the following week throughout the whole area; Quatsino Sound area (Forward Inlet and Winter Harbour), June 6.

First knowledge of the presence of immature pilchards on the east coast of Vancouver Island resulted from sighting a school on May 20 in Departure Bay, part of which was later captured in a beach seine. Subsequent information indicated that similar schools had been observed earlier in the area as follows: Victoria area (Brentwood),

early in May, and (Sooke), by mid May; Cowichan area, middle of May; Comox area, throughout the month of May.

North-east of Vancouver Island pilchards were first observed in Johnstone Strait on June 5.

In the northern part of the province first records of occurrence were: Smith Inlet, August 21; Calvert Island, August 18; Bella Bella, July 27; Milbank Sound, middle of July; Otter Channel, September 5; Pacofi, "end of September" according to the fisheries officer's reports, but a single specimen sent down from that port for identification was labelled "Sep.?" and was acknowledged on September 13.

In summary: Early in May pilchards were observed around the southern shores of Vancouver Island and in Barkley Sound. Later, they were observed progressively more to the north-west both in the Strait of Georgia and in the inlets on the west coast, reach Quatsino Sound and Johnstone Strait during the first week of June. Breaks occur in the regular progression of records in Queen Charlotte Sound and to the northward but it may be noted that the fisheries officer's report of occurrences at Smith Inlet and Calvert Island definitely indicate that the dates given are the dates of his personal observations rather than those of first reports.

Places where pilchards were observed during 1940

On the west coast of Vancouver Island pilchards were found generally distributed in all the principal sounds and inlets. Off-shore they were very abundant off Clayoquot and Barkley Sounds and as far as fifty miles off the states of Washington and Oregon. Around the southern and eastern coasts of Vancouver Island, they were reported from many locations between Sheringham Point and Cape Lazo. On the mainland shore they were recorded from the south side of the Strait of Juan de Fuca, Puget Sound, and in the Strait of Georgia from Burrard Inlet to Pender Harbour.

North of Vancouver Island, small pilchards were recorded from scattered locations between Helmchen Island and the south end of Pitt Island. There were two records from the Queen Charlotte Islands.

Young pilchards were reported as not being present from the following locations: northern part of the Strait of Georgia around Cape Mudge and in the various channels entering the north end of the strait; Grenville and Principe Channels; Bella Coola; Skeena River area; Naas River area. It may be noted here that there is a fairly close correspondence between the limit of distribution of the

small fish and the meeting place of the flood tide currents in the Strait of Georgia, and that the flood tide currents in Hecate Strait meet in the neighbourhood of Browning Entrance.

The preceding summary of the distribution of young pilchards was based upon the following and other records, most of which have been supplied by fishery officers:

West coast of Vancouver Island: All around Barkley Sound; all the inlets of Clayoquot Sound and Sydney Inlet full of pilchards; majority of the bays in Nootka Sound and Esperanza Inlet; Port Langford; Port Eliza; Muchalat Arm; along open beaches on the east shore of Brooks Peninsula; Naspate Inlet; Fair Harbour; Malksope Inlet; Southeast Arm; West Arm; Marble Creek; Rupert Arm; Klas-kino Inlet; Klaskish Inlet.

South end and east coast of Vancouver Island: Sheringham Point; Sooke; Becher Bay; off Esquimalt and Victoria; the Gorge, Victoria; Discovery Island; Haro Strait; Sidney; Canoe Cove; Saanich Inlet; Fulford Harbour; Tent Island; Kuper Island; Telegraph Harbour; Thetis Island; Chemainus; Ladysmith; Kuleet Bay; Nanaimo; Bal-lenas Islands; Oyster River; Baynes Sound; Comox.

Mainland coast: south side of the Strait of Juan de Fuca; Puget Sound; San Juan Islands; Burrard Inlet; Bedwell Bay; Fishermans Cove; Eagle Harbour; Keats Island; Gibsons Landing; Bargain Harbour; Pender Harbour; Secret Cove; Jervis Inlet; Salmon Arm.

North of Vancouver Island: Helmchen Island; Johnstone Strait; between Port Neville and Port Harvey; Cracroft Island; Baronet Passage; Blackfish Sound; Retreat Pass; Hardy Bay; Seymour Inlet; Kingcome Inlet; Knight Inlet below Hoeya Sound; Smith Inlet; Rivers Inlet; Bella Bella; Evans Arm; Caamaño Sound; Surf Inlet; Bishop Bay; Otter Channel; Pacofi; Selwyn Inlet.

The distribution indicated by the direct observations is largely confirmed by food studies made on spring salmon by Pritchard and Tester (1941) who report concerning the distribution of young pilchards: "None were taken off the northern end of the Queen Charlotte Islands, or in the northern part of the Strait of Georgia. Relatively few were recovered from the stomachs of Kyuquot and Quatsino, but many appeared from Queen Charlotte Sound to Banks Island, in the Barkley Sound area, and in Cowichan Bay."

Disappearance of young pilchards

An outward movement of young pilchards from the inlets of the west coast of Vancouver Island began in the latter part of August. By the end of October for Quatsino Sound and the end of September in the other areas, all the pilchards had left the inlets. Apparently they did not all go very far since in January, 1941, lingcod fishermen reported immature pilchards from six miles off Clayoquot Sound. On the east coast of Vancouver Island no such general exodus appears to have occurred. To the north of Vancouver Island herring fishermen made catches of young pilchards in Roscoe and Seymour Inlets during the winter but there is no information as to whether they remained in the inlets generally.

Associated species

Other species of fish were frequently observed with schools of pilchards either as associates or predators. Young anchovies were the most frequent associates in the Canadian inlets. At some times and places, they were found to constitute as much as 65 per cent of the mixed schools. These mixed schools were not stable as the two species sometimes separated entirely leaving almost pure schools of pilchards or anchovies. When such separations took place the schools of anchovies kept closer to the beaches than the pilchard schools. Pilchards were somewhat larger than young anchovies and the size discrepancy increased with the advance of the season. Anchovies were predominant in Sydney Inlet but reports from north of there on the west coast of Vancouver Island do not mention them as present and it is assumed that they were not abundant. They were, however, mentioned in reports covering Fitzhugh Sound at Evans Arm.

Young herring and young mackerel were also associated with young pilchards to varying degrees. The young herring were usually smaller than the pilchards. The mackerel were both longer and more robust and were readily recognizable in the water both by their size and by their vermiculate markings. Among some schools in Clayoquot Sound, mackerel constituted 50 per cent, but they were usually much more sparsely distributed. Herring were recorded as being mixed with pilchards (and anchovies) in Barkley Sound and northern British Columbia, and mackerel throughout southern British Columbia waters.

Salmon, rockfish, and marbled sculpins were observed feeding on the schools of young fish.

Abundance and uses

Accurate estimates of absolute abundance of free fish are difficult under any circumstances. In the present case, difficulties were increased by the novelty of the fishery which impaired the accuracy of fishermen's and fisheries officers' estimates, and by the varying mixture of the pilchards with anchovies.

The number of schools was very great and many would be sighted in the course of a day's travel. Fisheries officers in Kyuquot and Nootka areas estimated 3,000-5,000 tons and about 2,000 tons respectively as the amounts of fish in their areas but the latter remarks that fishermen in the area consider the quantity to be much higher. In Barkley Sound, 2,833 tons of mixed pilchards and anchovies were taken of which at least 50 per cent were estimated as being small pilchards. On the east coast of Vancouver Island, one operator took 676 tons of young pilchards and others unknown amounts without causing any apparent diminution of the supply. The total amount of young pilchards in the inshore waters of Canada was probably around 30,000 or 40,000 tons. How much was outside cannot even be estimated.

Most of the young pilchards captured were reduced along with whatever anchovies accompanied them. Some were canned and, in the case of one producer at least, an exceptionally fine product was put up. The pilchards were also used as live and dead bait and were very effective. They filled a large and undetermined role in the food cycle of large commercial fish. Pritchard and Tester (1941) show the large change which took place in the food of spring salmon in areas where the small pilchards occurred. Fishermen have remarked that, during the autumn and winter of 1940, spring salmon in the Strait of Georgia were numerous and in good condition. In certain localities young pilchards were common items in the food of lingcod.

Mortality of young pilchards during 1940 and the following winter

During the summer of 1940 young pilchards showed tendencies to strand on beaches or to enter fresh water and die. As early as May 25 an inspector reported: "At Chemainus thousands of pounds of these fish which had schooled up behind a boom of logs were left on the beach when the tide went out. . . . The same thing happened at Clam Bay when fish went up on the flats at high tide." In a report for the week ending August 31 the inspector for Quatsino area states: "During the early part of the week, large schools of immature pilchards were present in the lower part of Marble Creek, almost as far upstream as

the limit of tide water. Later in the week, thousands of these fish were lying dead on the bottom of the stream, and I presume that the fresh water was the cause as there were no marks of injury and the depth of water precluded the possibility of stranding during low tides." At the mouths of other streams in the area stranding was reported as taking place. A situation similar to that at Chemainus arose at Pender Harbour, where many tons of pilchards died, trapped by falling tides on the oyster beds at the head of the harbour. Other instances were reported at Long Beach on Texada Island and at Secret Cove. Similar cases of large-scale stranding are mentioned by Walford and Mosher (1941) at the mouth of the Columbia River. It would appear that all such cases are the result of some failure in adaptation of a pelagic fish to life off sheltered beaches.

During the winter 1940-1, an epizootic began in some localities which gave every indication of being different in kind from mortalities previously noticed. This mortality was the subject of a full report by the staff of the Pacific Biological Station (Foerster, 1941) from which much of the following is obtained. The report was based on studies in Finlayson Arm of Saanich Inlet, where at least 50 tons of fish died, and at Pender Harbour, where mortality was also heavy. Heavy mortality occurred at Todd Inlet. Other cases of mortality of less intensity were reported from Union Bay, Cowichan Bay, at Finnish Nook in Rivers Inlet, and in the Bella Bella area. Mortality was not limited to Canadian waters and reports indicate mass deaths in Puget Sound, near the San Juan Islands, and off Port Angeles in the Strait of Juan de Fuca. During January and February, while the epizootic was taking place, pilchards were plentiful in other parts of the Strait of Georgia area such as Ladysmith area, Lasqueti Island, and Nanoose Bay, and showed no sign of excessive mortality.

The syndrome of the epizootic was dead fish on the bottom, sluggish movement on the part of living schools swimming near the surface with "individuals occasionally turning on one side, circling for a few moments and then coming to the surface moving about with mouth gaping. . . . Some specimens were seen swimming about with the head turned sideways to the body as if partially paralysed." Individual fish, on examination, were found to be very thin. They showed well-developed or incipient red haemorrhagic areas on the surface and in the most marked cases blood oozed out from beneath apparently uninjured scales. Autopsy showed a red congested digestive tract and a green liver, but the latter symptom was not observed at Pender Harbour.

The explanation of the mortality offered by Foerster is that a specific dietary deficiency or deficiencies disrupted metabolic balance, leading to the production of toxic substances either directly, or by facilitating the ingress of bacteria. Final death was considered caused by failure in function of the liver or by failure in respiration due to extensive haemolysis and haemorrhage. It would appear that the heavy mortality is associated with the inability of a species which depends largely upon phytoplankton for food to maintain well-being under winter conditions in Canadian waters.

Growth of the 1939 year-class

Detection of growth of pilchards during the course of a year has not been possible in British Columbia as the fishery depends upon a population of mixed year-classes of older, slow-growing fish. Observations on the growth of the young fish which entered Canadian waters in 1940 have been possible as the fish were readily distinguishable by their size.

The size of the small fish when they first appeared marked them as beginning their second year. This diagnosis was confirmed by scale examinations on numerous samples. The fish accordingly belong to the 1939 year-class.

In Table IV median length is shown rather than average as the median is less affected by the inclusion of occasional extraneous specimens in the array. The limits of the interdecile range are also shown, to indicate the length spread in the samples. The median lengths of the samples are shown graphically in Fig. 1. Among other length differences reflecting differences in the history of the schools, the small size of the Barkley Sound specimens in 1941 will be observed. The smallest fish are of the 1940 year-class and do not come into consideration of the growth rate but the evidence indicates that the samples of larger fish are mostly of the 1939 year-class with a mixture of 1940 year-class fish. The June 1 sample showed a bi-modal frequency with separate medians at 104 mm. and 145 mm. The June 10 and 17 sample, where measurements suspected of being for 1940 year-class fish are omitted, gives a median of 156. It will be noted that the pilchards sampled in Barkley Sound during 1940 were of small size. During the commercial pilchard fishery of 1941, a noteworthy group of small fish with a modal length around 190 mm. was encountered and identified as the 1939 year-class. In the following year, fish around 210 mm. standard length were unusually abundant in commercial catches but these could

TABLE IV
REPRESENTATIVE LENGTHS FOR SAMPLES OF YOUNG PILCHARDS

			Standard lengths in millimetres			
			No. of fish	Lower decile	Median	Upper decile
1940						
May	17	Kildonan Harbour	50	90	97	105
May	17	Vernon Bay	100	90	95	103
May	20	Departure Bay	46	100	106	112
June	17	Green Cove, Barkley Sound	399	101	105	111
June	17	Green Cove, Barkley Sound	400	99	103	108
June	17	Green Cove, Barkley Sound	232	99	104	108
July	21	Barkley Sound	144	114	119	127
July	21	Barkley Sound	143	114	120	128
July	22	Off Oregon coast	50	131	137	146
Sept.	10	Cowichan Bay	204	137	145	153
Sept.	13	Spiller Channel	180	127	133	140
1941						
Jan.	6	Saanich Arm	94	135	150	170
Jan.	8	Pender Harbour	49	145	158	170
Jan.	27	Brentwood	119	138	150	167
Feb.	22	China Hat	51	150	160	170
March	9	Kendrick Arm, Nootka Sound	52	142	167	196
June	1	Barkley Sound	67	103	141	155
June	2	Sydney Inlet	49	138	160	182
June	2	Barkley Sound	49	105	110	119
June	7	Nootka	30	157	170	183
June	8	Hesquiat Bay	38	161	172	187
June	14	Barkley Sound	60	109	117	152
June	10)					
	17)	Barkley Sound	50	112	144	166
June	20	Pender Harbour	143	188	198	210
June	23	Nuchatlitz Inlet	22	158	180	188

be identified with the 1939 year-class with less confidence. The median lengths for small samples of pilchards taken at Sechelt Inlet on June 6, 1942 and at Nanoose Bay on January 4, 1943, were 208 mm. and 224 mm. respectively. It is assumed that these fish were of the 1939 year-class.

During the first thirteen months in Canadian waters, young pilchards grew from about 100 mm. standard length to an average of 170 mm. in standard length. If the form remained constant this should

represent an increase in weight of about 400 per cent corresponding to an increase in weight during the year from 10 grammes to 50 grammes. This is fairly well in keeping with observation. If 190 mm. is regarded as the appropriate length to consider, owing to its being obtained from material taken in the general fishery, an increase of nearly 600 per cent may be estimated and again this is in reasonably close agreement with the weights obtained from the samples.

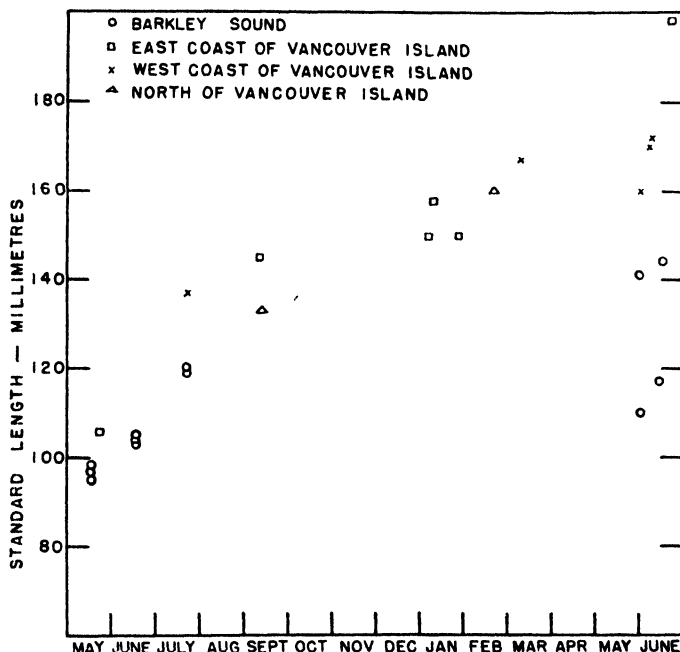


FIGURE 1.—Median lengths of pilchard samples

Subsequent history of the 1939 year-class in Canadian waters

In the summer of 1941 pilchards of the 1939 year-class were encountered as III-year fish in large numbers in Canadian waters. Their distribution appeared to be fairly general. In the commercial fishery on the west coast of Vancouver Island, which amounted to more than 50,000 tons during the regular summer season, small pilchards constituted rather more than 25 per cent of the individual fish, but their economic importance was less than that owing to their small size and consequent low individual weights. They were present on the east coast of Vancouver Island in the Strait of Georgia, where about 100 tons of survivors from the previous winter's mortality were captured.

They were also present in the Queen Charlotte Strait area to an unknown extent and in northern British Columbia, where they provided about 45 per cent of the individual pilchards taken, all aggregating more than 6,000 tons. This includes catches made on the Queen Charlotte Islands where they were reported as being quite abundant in the inlets of the west coast.

In the winter of 1941-2, considerable catches of pilchards were made off the Vancouver Island coast. Nearly 80 per cent of them were of the 1939 year-class. During the winter of 1941-2, some mortality occurred among pilchards remaining in the Strait of Georgia. Comparatively light mortalities were observed at Deep Bay and at Fanny Bay off Baynes Sound, which the local fisheries officer did not consider to be the results of stranding. At the head of Pender Harbour, many were found dead on the oyster grounds but it seemed likely that these were killed by stranding when the tide fell. During the winter of 1941-2, pilchards were taken to a significant extent in herring catches made in Khutzeymateen Inlet.

Pilchards of the 1939 year-class were prominent in the fisheries of 1942 but it is difficult to assess the exact importance in many cases where the length frequency distributions of the IV-year fish overlap those of the older year groups. In the usual fishery off the west coast of Vancouver Island, the 1939 year-class appeared to be of more relative importance than in the previous year and in the winter fishery off Vancouver Island (1942-3) and in the summer fishery farther north, 1939-year-class fish were predominant. In the Strait of Georgia and in the Queen Charlotte Strait areas, small commercial catches of 260 tons and 93 tons, respectively, consisted almost entirely of 1939-year-class fish. It cannot be assumed that the IV-year fish which were present in the west coast fishery were survivors of the original schools which were in the inlets and immediately off-shore during the summer of 1940. It is likely that the general Canadian fishery in 1942 drew upon 1939-year-class fish produced all over the north-east Pacific, but that the Strait of Georgia catches were dependent upon the survivors of two summer fisheries and two winter mortalities.

A few small schools of pilchards are still observed in the Strait of Georgia area but it is evident that the pilchard fishery in that area is no longer of economic significance. In the regular fishery on the west coast of Vancouver Island, the part played by 1939-year-class fish will no doubt continue prominent and even increase.

DISCUSSION

The group of II-year pilchards which entered Canadian waters in 1940 was beyond question the most abundant group of small fish of the species to appear off the Canadian coast within the last twenty-five years and probably considerably more. Many of the unusual, apparently qualitative features of its behaviour depend primarily upon quantity. Ubiquitous distribution is an expected result of increased abundance. The winter fishery, depending to a large extent on young individuals, may seem less remarkable in view of the fact that young pilchards have been recorded before in Canadian waters during the winter and if they had been more numerous could have supplied a fishery. On a previous occasion, small quantities of young pilchards which were present in Vancouver Island inlets during the spring left during the summer, as did the larger bodies during the summer of 1940. The association with anchovies is a regular occurrence in regions where both species are present in abundance. The 1939 year-class was unique in that it was recognizable for several years, and this too can well be associated with the abundance of the small fish as compared with that of the older age groups. Even the phenomenal mortality is associated with abundance. Foerster (1941) suggests that the size of the pilchard population in Saanich Inlet may have been great enough to aggravate the food shortage by depleting the plankton crop and may thereby have increased the mortality. Whether this suggestion is correct or not, it is reasonably certain that a mortality which involved one-one-hundredth or even one-twentieth of the number of deaths would have remained only a matter for passing comment.

It would appear that fundamentally the influx of pilchards and its associated occurrences depended upon the marked success of a northern spawning as deduced also by Walford and Mosher (1941). A spectacular failure of the Canadian pilchard fishery during 1939 would suggest that this spawning took place either well off-shore or south of the area reached by scouting operations of the Canadian pilchard fleet. When the pilchards resulting from the spawning were about one year old they came in-shore first approaching the coast of Vancouver Island below Clayoquot Sound, spreading along the southern coast into the Strait of Georgia and north along the shore into most of the inlets including Queen Charlotte Strait, and beyond Vancouver Island as far as the south end of Pitt Island. South-east of Barkley Sound and off the United States coast large bodies of young pilchards remained off-shore.

It is noticeable that the limits in the inside passages of the first year's dispersion in a northward direction correspond fairly closely with the limits of northward-flowing flood tides, but carriage by flood tide currents cannot explain the failure of the small pilchards to enter the Strait of Georgia through Johnstone Strait. The migration through the long narrow channels may have been too long and it is noticeable that records of pilchards during 1940 were not received for locations near the heads of such long channels as Bella Coola, Knight Inlet, and Jervis Inlet.

It is possible that the advent of small pilchards in Canadian fishing grounds is a premature indication of what may be expected as a result of the continued intensive fishing for the species. Other intensive fisheries have experienced the change from exploiting large mature fish to pursuing younger smaller ones, and the Canadian pilchard fishery has been depending upon populations of successively smaller fish each year since 1937. It seems unlikely that such a radical change would take place suddenly and more probable that the appearance of the 1939 year-class in northern waters was the result of particularly favourable oceanographic conditions.

There is a coincidence between the occurrence of small pilchards on the west coast of Vancouver Island and the presence of pilchards of some kind north of Vancouver Island. They have been reported as abundant north of Vancouver Island in the five years: 1931, 36, 40, 41, and 42. In each of these years, either notably small pilchards were abundant on the Vancouver Island coast or the average size of the main group was smaller than in the preceding year.

The policy to be recommended in connection with exploiting young pilchards in Canadian waters depends upon several opposing considerations. It has been shown earlier that, during the first year of availability, on the average a young pilchard increases in weight (and accordingly in value) to five or seven times its original size. As it seems unlikely that, barring catastrophe, mortality would amount to 80 or 86 per cent during the year, it would appear profitable to leave the young fish to grow for at least one additional year. However, at the present time there is no assurance that catastrophe in the form of an epizootic is not the normal event to be expected in the Strait of Georgia. Until that assurance is provided by experience, it seems best to permit full exploitation in that area. On the west coast of Vancouver Island, restriction, probably on the basis of international co-operation, would appear indicated.

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INTRABRONCHIAL FIXATION OF THE HUMAN LUNG FOR PURPOSES OF ALVEOLAR MEASUREMENT, USING 25 μ MICROSECTIONS MADE THEREFROM¹

By W. STANLEY HARTROFT and CHARLES C. MACKLIN, F.R.S.C.

A PRACTICAL method for the preservation of mammalian lungs at a controlled degree of expansion is essential if they are to be used for determination of the size of their alveoli from their microsections. Fixation by immersion and by acupuncture are ruled out because the alveoli are then unequally distended or collapsed. Vascular perfusion of human lungs in the intact thorax is not usually feasible, for several reasons, e.g. the process requires much time to effect the thorough stiffening of the lung substance so necessary for the retention of alveolar shape and size in microsections. Bronchial filling, with Bouin's picro-formol solution (1), of the isolated, collapsed, normal, human lungs immersed in a bath of 3 per cent formalin, with control of the degree of expansion by conformity of lung length to definite pleural cavity dimensions of the same thorax, has proven satisfactory. The resulting lung tissue is evenly distended, and the stage of expansion is generally comparable with that of the lungs of the embalmed cadaver, and also (with reservations) with that of the lungs of experimental animals fixed by vascular perfusion with intact thorax. This last point is important, for the data on alveolar size derived from these lungs will, in a later paper, be compared with those from the common laboratory animals fixed by the last-mentioned method.

The thoracic cavity is opened as soon after death as possible by reflecting the skin and subcutaneous tissue outward from a midline incision. The anterior thoracic wall is cut along the costochondral junctions and the sternum and attached cartilages removed. The heart and lungs are taken out *en masse*, avoiding pleural perforation. Notes are made on lung points such as size, weight, and appearance.

The pleural cavity control measurements. The most important step in this method is the taking of certain control measurements from the interior of the pleural cavity with which corresponding dimensions of the lung are brought into equality as bronchial filling progresses to establish the desired degree of expansion. The dimension taken is the dis-

¹This work was done under grants from the National Research Council of Canada and the Department of National Defence, Canada.

tance between two points which, in the living body, are contiguous to the extremities of the lung. The superior point (the cupola) is the caudal border of the neck of the first rib. The inferior point is the lower border of the eleventh rib at the costovertebral junction. The measurement is taken on both sides with obstetrical calipers, flexed in reverse, just lateral to the vertebral column. No allowance is made for the slightly greater length of the left lung as compared with the right, for it is felt that the difference in level of the two lungs is not as great as the margin of error involved in the measurements. J. S. Dickey (2) has illustrated this relation for both right and left pleural cavities in his figures 37 and 38. This measurement is taken to correspond with the superior-inferior dimension of the expanded lung from its apex to the paravertebral part of its inferior border, and thus it gives a standard of length to which each lung must be brought when being filled with fixative. When so distended it is felt that these lungs are justifiably comparable in degree of expansion with those of our experimental animals which have been fixed by vascular perfusion with the thorax intact. For instance, the lower border of the eleventh rib was found to be the level of the inferior (caudal) end of the paravertebral ridge of a baboon's lungs, fixed *in situ*. They are also in close agreement with those of cadavers used for topographical anatomy.

Filling the lungs with fixative to the required size. A glass cannula is promptly affixed in the trachea and, with the fresh lungs immersed in a bath of 3 per cent formalin to provide adequate support to all parts while being filled, a small quantity of fixing fluid is poured into the cannula *via* a funnel and allowed to run into the bronchial tree. The preparation is gently moved about and air bubbles allowed to escape. This process is repeated many times, the residual air being largely replaced by the fixing fluid. When bubbles no longer emerge, a rubber tube and pressure bottle, filled with fixing fluid, are attached to the cannula, and the inflow resumed at a pressure of approximately thirty inches of water. A close check, by means of calipers, is kept on the greatest length of each lung, as already described, along the paravertebral ridge, and when this dimension exceeds by 15 per cent the pleural cavity control measurement previously ascertained, the inflow is stopped. It is necessary to overfill human lungs to this extent to provide for the inevitable shrinkage which follows loss of fluid through the visceral pleura. This shrinkage is even more marked in the bronchially filled lungs of smaller animals, such as the rabbit, than in those of man. After this shrinkage has occurred, the length of the lung cannot be

restored through later injections of fixing fluid, and attempts to do so result in ruptures of the stiffened tissue. Each lung is made to enlarge to agreement with its own dimension. When the desired degree of distention has been attained, the tubing is clamped off. Such a preparation should lie in the supporting bath for three or more weeks with checking of length from time to time until the lung tissue is so stiff that it will hold its shape in the processing operations and will faithfully reflect alveolar size in the microslides save for unavoidable shrinkage in dehydration.

Selecting and excising the blocks. With the aforementioned laboratory animal lungs it is a simple matter to cut a block from each of the seven lobes; but for the human lungs this is more difficult because not all of these lobes are marked off by fissures. It is desirable to select blocks from the human lungs in a manner resembling as closely as possible that adopted for animals' lungs, in order that the determinations of the alveolar size in both animals and humans might be based on similar methods of sampling. To facilitate the exact localization of these blocks so that they correspond with those of the animals, the human lungs are first cut carefully, with a brain knife, into transverse slices 3 centimetres thick. This operation is done under 3 per cent formalin with a special miter box. To ensure that the slices are exactly parallel the sections are made at right angles to a line, marked on the surface of the lung with an electric cautery, and extending from the apex to a definite point on the inferior border, midway between its anterior extremity and the rounded paravertebral ridge.

When these slices are laid out under 3 per cent formalin, it is possible to recognize on their cut surfaces the main bronchi and their principal branches. A map of these parts of the bronchial tree may then be drawn, and the lobar regions, not specifically separated (left middle and right cardiac lobes) identified.

The blocks representing the right and left upper lobar regions were taken from the postero-lateral aspects of the costal surfaces of each lung, at a level approximately 6 centimetres below each apex. The right and left middle lobar regions were represented by blocks selected from the postero-lateral aspects of each lung approximately 15 centimetres below each lung apex. These blocks were taken, on the right, from the naturally occurring right middle lobe, and, on the left, from the lower portion of the left upper lobe. The right and left lower lobar regions were represented by blocks taken from the postero-lateral aspects of the right and left lower lobes respectively, at a level approximately 6 centimetres

metres below the apex of each hole. The block representing the cardiac region was also taken at a level 6 centimetres below the apex of the right lower lobe, from the anterior aspect of its mediastinal surface which had been in apposition to the right side of the heart and the inferior vena cava.

For the identification of the region of the human lung corresponding to the cardiac lobe of animals, the specially made stereophotographs kindly sent to one of us (C.C.M.) by Dr. John Franklin Huber (3) of the Department of Anatomy of Temple University, Philadelphia, Pa., were very helpful.² These were of a human right lung possessing the rare anomaly of an almost completely separated cardiac process. By comparing the bronchial pattern of Huber's right lung with the maps of the bronchial trees of our expanded right lungs we were able to locate exactly that part of each of our lungs which was supplied by a bronchus corresponding to the one going to the cardiac lobe of Huber's specimen, and with this guidance a block representing the cardiac lobe was located precisely and excised.

Preparing the sections. The blocks are dehydrated in ascending strengths of ethyl alcohol and embedded in paraffin according to a standard routine used for human and animal lung tissue intended for determinations of alveolar size. Sections, 25μ in thickness, were cut and stained with hematoxylin and eosin, and measurements were made on outlines of alveoli appearing in them by a method to be described in another paper. These sections, and also those of other thicknesses cut from the blocks and stained in various ways, provide a basis of normality of human lung structure. They may be used in many different lung studies. It would be well if medical students were given the opportunity of acquiring a knowledge of the histology of the normal human lung from material preserved in this way, which avoids the disadvantages of over- and under-distention.

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²The authors wish to thank Professor Huber for his kindness in sending the excellent photographs and description of this interesting anomaly.

SCALE PATTERN AND SCALE COUNTING METHODS IN RELATION TO CERTAIN TROUT AND OTHER SALMONIDS

By FERRIS NEAVE

Presented by J. R. DYMOND, F.R.S.C.

INTRODUCTION

THE scales of salmonid fishes conform to a general type in structure and arrangement. Within the family, considerable variations occur in the number of scales present on the body. Such differences have been used rather extensively in characterizing genera, species, and smaller units. The purpose of the present paper is to discuss certain features in the development of the scale pattern and to consider the significance of different scale-counting methods in attempting to distinguish between certain species and populations.

MATERIAL

Most of the material was obtained during the course of experimental work at the Cowichan Lake Hatchery, Vancouver Island, and consisted of various populations of *Salmo clarkii* Rich., *S. gairdneri* Rich., and *S. trutta* L., both "wild" and hatchery-raised specimens being used. A few additional observations were made on Pacific salmon (*Oncorhynchus tshawytscha* Walb., *O. kisutch* Walb., and *O. gorbuscha* Walb.) and chars (*Salvelinus fontinalis* Mitch. and *S. malma spectabilis* Gir.).

TECHNIQUE

In small fish it is difficult or impossible to appreciate the number and arrangement of the scales or scale papillae on the intact individual. The skin can readily be removed, however, from fish which have been fixed in formalin and the following procedure was adopted for small individuals. After wiping the mucus from the surface of the body, incisions are made with a sharp blade along the back, belly, and around the gill region of one side. The skin is then stripped off from head to tail (Fig. 1), placed on a slide and scraped on both sides with a safety-razor blade. This removes the underlying pigment and the scales. After a brief staining in methylene blue the wet skin is dried with a cloth and wiped from tail to head. This causes the scale pockets or scale papillae to stand out with great clearness. The removal and preparation of a skin in this manner usually require less than five minutes.

DEVELOPMENT OF SCALE PATTERN

In the species examined by the writer (*S. clarkii*, *S. gairdneri*, *S. trutta*, *S. fontinalis*, *O. tshawytscha*, *O. kisutch*) the first scale papillae to appear occur beneath the sense organs of the lateral line. At or about the time of hatching these sense organs are present as a single series along the side of the trunk and tail. Throughout most of the length of the body each is situated just behind a myoseptum. At the posterior end of the series, however, the myomeres are indistinct or not yet estab-

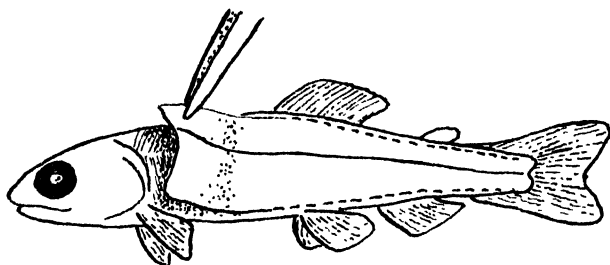


FIGURE 1.—Young salmonid, showing method of removing skin.

lished. After a more or less lengthy period some of the sense organs divide, giving off a neuromast which lies dorsad to the original organ and may become widely displaced from it. About the same time or somewhat later new organs arise along the line of the original series, alternating with the original members. Thus the number of sense organs in strict lateral line series becomes doubled.

No scale papillae are evident until these developments have taken place. Papillae then appear beneath the organs of the completed lateral line series, those which arise beneath the newer, interstitial organs being frequently smaller than the others for a considerable time. Alternation of larger and smaller papillae was observed by Elson (1939) in the speckled trout, although the association with sense organs was not indicated. Formation of papillae spreads dorsally and ventrally from the lateral line by means of oblique lines of papilla-forming cells extending from the centres of development on the lateral line (Neave, 1936; Elson, 1939). In the posterior body region of *Salmo* each lateral line papilla commonly gives rise to one dorsal and one ventral oblique outgrowth. In *Salvelinus*, however, and to a varying extent in the anterior region of *Salmo*, the outgrowths may be double from their starting point and/or may branch later, thus increasing the number of oblique scale rows. Extinction of oblique lines may also occur but since this is less common than branching, the number of scales in horizontal rows is usually greater

(sometimes very much greater) at levels above and below the lateral line than on the line itself.

At the extreme caudal end of the body, scale formation both on and outside the lateral line is considerably delayed.

METAMERISM

As indicated above, the original scale papillae of the lateral line, in the species discussed, are distributed with a high degree of metameric regularity throughout at least the greater part of the body. The typical relationship in position between sense organs, papillae, and myosepta is shown in Fig. 2. From the data supplied by Foerster and Pritchard (1935) regarding number of lateral line scales and vertebrae in *Oncorhynchus*, it is evident that the ratio of two scales per metamere is maintained, at least approximately, in *O. tshawytscha*, *O. kisutch*, and

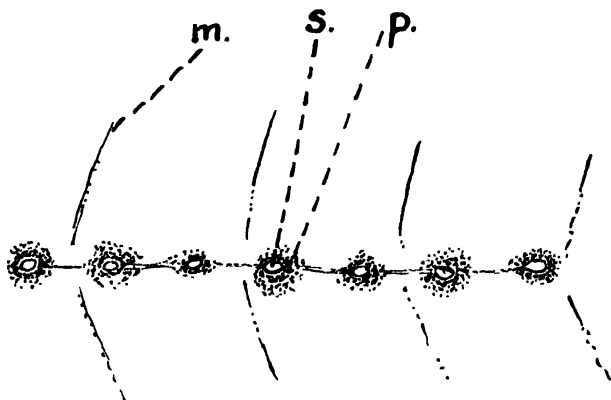


FIGURE 2.—Portion of lateral line region of a cutthroat trout, 37 mm. long, showing relation between sense organs (s), scale papillae (p), and myoseptum (m) $\times 48$.

O. nerka of a somewhat more advanced age. In the pink salmon (*O. gorbuscha*), however, the number of scales is greatly in excess of this ratio (average number of scales 175.2, average number of vertebrae 66). After examining a few small pink salmon fingerlings the present writer believes that the first scale papillae show the same distribution as in other species but that subsequently papillae develop between the primary members of the lateral line series, as well as dorsad and ventrad to the latter. This development can perhaps be correlated with the comparatively large size attained by this species before scale formation begins, resulting in a wider spacing between the sense organs and thus leaving room for the establishment of papillae. (Spatial considerations also

appear to be of importance in the establishment of papillae in certain cases of regeneration (Neave, 1940).) Appearances suggestive of similar interpolation are occasionally seen on the lateral line of trout.

The scales which develop above and below the lateral line appear to be quite independent of the metamerism of the body.

METHODS OF SCALE COUNTING

In attempting to compare the scale patterns of different fish, it is customary to count the number of scales between certain points on the body. Scale-counting methods in current use in North America include:

- (a) Counting of scales in the lateral line series from the gill aperture to the "end of the vertebral column" (Foerster and Pritchard, 1935) or "base of the tail-fin rays" (Needham, 1938).
- (b) Counting of scales in the longitudinal row immediately dorsad to the lateral line series. This method is recommended by Foerster and Pritchard (l.c.) and Clemens (1935) for Pacific salmon.
- (c) Counting of oblique rows of scales between the points indicated above (Dymond, 1932; Mottley, 1934). In this method the count is not necessarily made at the same horizontal level throughout the body. On average, the level is usually considerably above the lateral line.

From the remarks made under "Development of the scale pattern" it would seem that a fourth method might be found in an estimate of the degree of branching exhibited by the oblique scale rows, as distinct from the total number of scales at a given level of the body. This could be expressed as:

- (d) Counting the number of oblique scale rows corresponding to a given number of lateral line scales.

APPLICATION OF METHODS

As a means of identifying individual fish, lateral line counts do not provide a clear-cut distinction between the various species of Pacific salmon, although the pink salmon can nearly always be separated from the four other species in this manner (Foerster and Pritchard, 1935).

Lateral line counts made by the present writer on various samples of trout are present in Table I. From these data it is evident that while the brown trout (*S. trutta*) examined are almost completely segregated by this means, lateral line counts afford very little basis for assigning individuals to the other specific and intraspecific groups represented. It may be added that a few specimens of *Salvelinus fontinalis* and *S. malma spectabilis* also yielded counts well within the range shown by *Salmo clarkii* and *S. gairdneri*, namely 124 to 129.

TABLE I

FREQUENCY OF SCALE COUNTS MADE ON THE LATERAL LINE

Salmo clarkii (1) = cutthroats reared at Cowichan Lake Hatchery.

(2) = cutthroats reared at Veitch Creek Hatchery, Vancouver Island.

Salmo gairdneri (1) = steelheads reared at Cowichan Lake Hatchery.

(2) = sea-run steelheads, caught in Cowichan River.

(3) = non-sea-run rainbow trout, caught in Cowichan River.

(4) = Kamloops trout, hatched and reared at Cowichan Lake Hatchery.

Salmo trutta = brown trout caught in Cowichan River and tributaries.

No of scales	<i>Salmo clarkii</i>		<i>Salmo gairdneri</i>				<i>Salmo trutta</i>
	(1)	(2)	(1)	(2)	(3)	(4)	
105	-	-	-	-	-	-	1
106	-	-	-	-	-	-	-
107	-	-	-	-	-	-	-
108	-	-	-	-	-	-	-
109	-	-	-	-	-	-	2
110	-	-	-	-	-	-	5
111	-	-	-	-	-	-	-
112	-	-	-	-	-	-	6
113	-	-	-	-	-	-	1
114	-	-	-	-	2	-	8
115	-	-	-	-	1	-	1
116	2	2	-	-	2	-	1
117	1	5	-	-	3	-	-
118	4	6	-	-	7	-	-
119	2	3	1	3	10	-	-
120	5	6	2	2	15	-	-
121	5	-	3	4	10	1	-
122	9	6	5	8	5	2	-
123	2	-	4	10	3	-	-
124	5	-	16	14	3	4	-
125	2	1	8	3	-	5	-
126	3	1	11	5	-	5	-
127	2	-	4	2	-	2	-
128	6	-	10	-	-	4	-
129	-	-	2	1	-	-	-
130	1	-	3	-	-	2	-
131	-	-	1	-	-	-	-
132	-	-	-	-	-	-	-
133	1	-	-	-	-	-	-
No. of fish	50	30	70	52	61	25	25
Mean	122.8	119.5	125.2	123.3	119.7	125.7	112.0

TABLE II

FREQUENCY OF COUNTS OF OBLIQUE SCALE ROWS ABOVE LATERAL LINE

No. of scales	<i>Salmo clarkii</i>		<i>Salmo gairdneri</i>				<i>Salmo trutta</i>
	(1)	(2)	(1)	(2)	(3)	(4)	
115	-	-	-	-	1	-	-
116	-	-	-	-	1	-	1
117	-	-	-	-	3	-	2
118	-	-	-	-	1	-	-
119	-	-	-	-	9	-	-
120	-	-	-	-	3	-	-
121	-	-	-	-	10	-	4
122	-	2	-	-	4	-	2
123	-	2	2	2	5	-	1
124	-	-	3	-	10	-	2
125	-	-	1	1	5	-	2
126	-	1	3	4	2	-	3
127	-	1	3	5	2	-	-
128	-	1	5	2	2	-	-
129	-	-	9	5	1	-	-
130	-	-	5	2	2	1	2
131	-	-	5	4	-	-	3
132	-	-	4	3	-	3	-
133	-	1	3	3	-	-	1
134	-	1	5	6	-	1	1
135	-	2	5	1	-	2	-
136	-	2	4	5	-	-	1
137	-	1	6	1	-	-	-
138	-	2	2	4	-	2	-
139	-	-	2	2	-	-	-
140	-	2	1	-	-	2	-
141	-	3	-	1	-	-	-
142	-	1	1	-	-	1	-
143	-	2	1	-	-	2	-
144	-	1	-	-	-	1	-
145	-	-	-	-	-	-	-
146	2	1	-	-	-	1	-
147	-	1	-	-	-	1	-
148	-	-	-	-	-	-	-
149	3	-	-	-	-	2	-
150	-	-	-	-	-	1	-
151	1	-	-	-	-	2	-
152	3	-	-	-	-	-	-
153	1	2	-	-	-	1	-
154	1	1	-	-	-	-	-
155	2	-	-	-	-	2	-
156	4	-	-	-	-	-	-
157	-	-	-	-	-	-	-
158	2	-	-	-	-	-	-

TABLE II (Continued)

No. of scales	Salmo clarkii		Salmo gairdneri				Salmo trutta
	(1)	(2)	(1)	(2)	(3)	(4)	
159	4	-	-	1	-	-	-
160	1	-	-	-	-	-	-
161	2	-	-	-	-	-	-
162	5	-	-	-	-	-	-
163	1	-	-	-	-	-	-
164	3	-	-	-	-	-	-
165	3	-	-	-	-	-	-
166	2	-	-	-	-	-	-
167	1	-	-	-	-	-	-
168	2	-	-	-	-	-	-
169	2	-	-	-	-	-	-
170	-	-	-	-	-	-	-
171	2	-	-	-	-	-	-
172	1	-	-	-	-	-	-
173	-	-	-	-	-	-	-
174	-	-	-	-	-	-	-
175	-	-	-	-	-	-	-
176	1	-	-	-	-	-	-
177	1	-	-	-	-	-	-
No. of fish	50	30	70	52	61	25	25
Mean	160.4	137.4	131.5	132.23	122.3	142.6	125.3

While certain recent authors have indicated that a high number of scales "on the lateral line" is characteristic of *Salvelinus* and *Salmo clarkii*, it is apparent that the figures quoted by them do not in fact refer to this scale row, but to counts made at a different level of the body. As shown in the table, the average lateral line scale count of cutthroats (*S. clarkii*) from Cowichan Lake Hatchery is actually lower than that of steelheads (*S. gairdneri*) from the same source.

Although of very limited value as a criterion for the identification of species, lateral line scale counts, used in a statistical manner, will sometimes serve to demonstrate differences between intraspecific populations. For example, in Table I there are significant differences of the mean value between rainbow and Kamloops trout and between rainbow and steelhead.

The possibility of finding a more satisfactory criterion at some other level of the body would obviously depend on the occurrence of specific differences in the degree of departure from the lateral line condition in the development of the scale pattern above or below the line. Foerster and Pritchard (1935) have shown that in the pink salmon the increase in

TABLE III
 FREQUENCY OF COUNTS REPRESENTING NUMBER OF OBLIQUE SCALE ROWS
 CORRESPONDING TO FIRST SIXTY SCALES OF LATERAL LINE

No. of scales	Salmo clarkii		Salmo gairdneri				Salmo trutta
	(1)	(2)	(1)	(2)	(3)	(4)	
60	-	-	1	-	3	-	-
61	-	-	4	2	9	-	-
62	-	-	4	1	10	-	-
63	-	1	3	5	8	-	-
64	-	1	4	2	14	-	-
65	-	1	10	4	10	-	3
66	-	1	6	4	3	-	-
67	-	-	4	3	2	1	1
68	-	-	7	7	1	1	2
69	-	2	3	6	1	3	1
70	-	-	4	5	-	-	1
71	-	-	7	1	-	3	5
72	-	1	6	3	-	3	-
73	-	3	2	3	-	3	1
74	-	-	4	2	-	2	3
75	-	1	-	2	-	4	4
76	-	2	1	1	-	2	1
77	-	4	-	-	-	1	3
78	-	2	-	-	-	1	2
79	1	3	-	-	-	3	1
80	1	1	-	1	-	2	-
81	2	1	-	-	-	3	-
82	2	2	-	-	-	-	1
83	4	1	-	-	-	-	1
84	2	-	-	-	-	-	-
85	4	1	-	-	-	1	-
86	-	-	-	-	-	1	1
87	5	1	-	-	-	-	-
88	4	-	-	-	-	-	-
89	8	1	-	-	-	-	-
90	6	-	-	-	-	-	-
91	4	-	-	-	-	-	-
92	4	-	-	-	-	-	-
93	4	-	-	-	-	-	-
94	3	-	-	-	-	-	-
95	2	-	-	-	-	-	-
96	3	-	-	-	-	-	-
97	-	-	-	-	-	-	-
98	1	-	-	-	-	-	-
99	-	-	-	-	-	-	-
100	-	-	-	-	-	-	-
101	1	-	-	-	-	-	-
No. of fish	61	30	70	52	61	34	31
Mean	89.0	76.1	67.4	68.3	63.4	75.2	73.5

the number of scales in the first row dorsad to the lateral line is so much greater than in the other species of *Oncorhynchus* that a clear separation of this species can be made. The writer has not applied this method extensively to trout, partly because the scales next to the lateral line are often irregular in disposition and doubt frequently arises as to whether a scale should be considered as belonging to a particular horizontal row. Observations on cutthroats and steelheads suggest that this procedure is somewhat less convenient than the following, although leading to rather similar results.

With references to the third method of scale counting (in the genus *Salmo*) Mottley (1937) says: "In western North America, however, it has become the established custom to count the number of oblique parallel rows of scales running in a downward and backward direction from the dorsum to the lateral line; the series begins just behind the head and terminates at the end of the vertebral column at the posterior reference point of the standard length measurement." He states further, "It has been the practice of the writer to make the count about ten or fifteen rows above the lateral line in the anterior part of the series, and beyond the adipose fin to continue the count at a level from five to ten rows above the lateral line." It is probable that considerable latitude exists in the practices of different systematists. While it would appear at first sight that this method is less precise than confining the count to a particular longitudinal row, it avoids to a considerable extent the difficulty mentioned in the previous method.

From Table II it can be seen that the counting of oblique scale rows does not provide a reliable means of distinguishing between any of the three species concerned. It does, however, provide a clear distinction between the cutthroats and steelheads reared at Cowichan Lake Hatchery. A complete or nearly complete separation is also apparent between this particular series of cutthroats and the brown trout and between the imported Kamloops and local rainbow trout. Significant differences of the means are also found in making certain other comparisons between these series.

The practice of counting oblique scale rows throughout the length of the body has certain disadvantages in common with the procedure discussed previously. (1) The caudal region of the series frequently permits of some latitude in interpretation both because of difficulty in determining the posterior reference point and because of irregularities in the oblique scale rows. (2) Since the posterior scales are late in developing, counts cannot be made on very small fingerlings. (3) These methods fail to take account of scale pattern, as distinct from mere

number of scales. For example, they fail to distinguish between a trout which yields a high scale count through virtue of possessing a large number of metameræ, and one in which the high count is due to more frequent branching of the oblique scale lines.

In order to obviate or diminish these difficulties, the writer has at times practised a method of counting the oblique scale rows corresponding to a portion of the lateral line. The procedure has been to count sixty scales along the lateral line, beginning at the anterior end, and then to count the oblique rows back to the head, beginning with the row corresponding to the sixtieth lateral line scale. In general the count was made about a quarter or a third of the distance from the lateral line to the dorsum. In the case of very small fish in which scale development has not advanced far, it may be necessary to descend to the first or second row above the lateral line at the extreme anterior end. In the writer's experience this has not seriously affected the results.

Table III shows the results of applying this method to the material previously considered (together with a few additional fish which were not sufficiently developed for making scale counts involving the caudal end of the body). In general the groupings obtained are similar to those which result from counting the total number of oblique rows, though the shift in position of the brown trout relative to the steelhead shows the greater amount of branching which takes place in the anterior region of the former species. There are indications, however, that the procedure may at times be more discriminating than the one previously considered, in addition to the advantages of its applicability to smaller fish and, in the opinion of the writer, the somewhat greater precision attained in counting. The cutthroats tabulated as from Veitch Creek include individuals from the brood years of 1937 and 1939. Taken separately, the 1937 fish yielded the following individual scale counts:

Total number of oblique rows	132, 135, 136, 137, 138, 138, 138, 140, 140, 141, 141, 143, 144, 145, 146, 153, 154, 156.
Oblique rows related to first 60 lateral line scales	75, 77, 75, 79, 79, 79, 79, 81, 78, 77, 82, 82, 80, 84, 83, 87, 89, 85.

According to Dymond (1932) the number of oblique scale rows in the steelhead or rainbow trout of British Columbia waters varies from 124 to 146 and in the coastal cutthroat from 143 to 180. Steelheads and cutthroats reared at Cowichan Lake Hatchery showed very similar ranges of variation, namely 123 to 143 and 146 to 177, respectively. The "first 60" counts for these hatchery fish were: steelheads, 60 to 76; cutthroats,

79 to 101. According to these three standards, the allocation of these Veitch Creek cutthroats would be as follows:

	Cutthroat	Intermediate	Steelhead
Total oblique rows (Dymond)	3	4	11
Total oblique rows (hatchery)	4	2	12
Oblique rows to 1st 60 1.1 scales (hatchery)	13	3	2

It would thus appear that in this particular series of fish the reduction in the total number of scales had not entailed an equivalent reduction of oblique scale rows in the anterior region.

DISCUSSION

It is evident that in the genus *Salmo* the range of variation in number of scales is least on the lateral line. When the various groups examined are combined as species, an extreme range of 18 scales is apparent both for *S. clarkii* (116 to 133) and *S. gairdneri* (114 to 131). This presumably represents a variation of about nine metameres in the architecture of the body. The smaller series of *S. trutta* showed a range of variation of 12 scales.

At a higher level on the body the range is much greater, amounting to 56 scales in *S. clarkii* (122 to 177), 45 scales in *S. gairdneri* (115 to 159) and 21 scales in *S. trutta* (116 to 136). In individual fish of all these species, the total number of oblique rows always equalled or exceeded the number of lateral line scales except in a very few *S. gairdneri*, in which counts of one or two scales less were recorded. A slight deficiency in oblique rows is more often apparent, however, if attention is confined to the posterior half of the body, where the individual scales are larger.

While, therefore, the number of lateral line scales appears to fix an approximate *minimum* figure for the number of oblique rows, the actual number of the latter which develop may exceed this minimum to a small or large degree. Determination of the number of oblique rows apparently takes place at a different time from, or under the influence of other factors, than determination of the number of lateral line scales. Thus, the series of Kamloops trout show an average lateral line count almost identical with that of hatchery-reared steelheads, whereas the average "oblique rows" counts differ by 11 scales. An aberrant sea-run steelhead yielded a count of 159 oblique rows, about 27 rows higher than the average of its group, although its lateral line count of 126 was less than one above the mean.

In comparing species, some degree of correlation can be found between the number of oblique rows and the size of the fish when these

rows are developing. In *S. trutta* and *S. gairdneri*, which usually possess fewer rows, these begin to develop (in individuals examined by the writer) when the fish is about 26 to 30 mm. long (standard length), whereas in the more finely-scaled *S. clarkii* and *Salvelinus fontinalis* the same degree of development is deferred until a length of 32 to 39 mm. has been reached. The similar distinction between the pink salmon and other species of *Oncorhynchus* has already been mentioned. Indeed the difference between species in the degree of scale development at equivalent sizes is sometimes a helpful indication in identifying very small trout.

It is evident from the samples discussed that while one or more methods of scale counting may serve to separate *S. clarkii* and *S. gairdneri* belonging to certain populations or in a given locality, no very useful distinction of this sort can be drawn between these species in general at the present time, even if *S.g. kamloops* is eliminated from consideration. Attention may be drawn to the much lower scale counts recorded for cutthroats than are usually credited to this species. In each species significant average differences may occur between the scale counts of different populations.

With regard to the influences which determine variations in number of scales, external conditions, including temperature, no doubt play a part (Mottley, 1934). There is, however, strong evidence (Neave, MS) that hereditary factors are concerned in the production of the scale pattern in certain populations of *S. gairdneri*.

SUMMARY

The development of the scale pattern in certain species of *Oncorhynchus*, *Salmo* and *Salvelinus* is described and discussed.

Scale development begins after establishment of the series of lateral line sense organs, under each of which a scale papilla develops.

These papillae tend to be metamerically distributed. Departures from the metameric condition are due, in some cases at least, to interpolation.

At levels dorsal to the lateral line the scales depart more or less widely from the metameric condition. Counts made at such levels are usually higher than lateral line counts, the increase in number of scales being mainly or entirely on the anterior part of the body.

Variations in number of scales are less extreme on the lateral line than at higher levels of the body.

The lateral line scales and non-lateral line scales may vary in number independently.

Considerable differences between the mean scale counts of different intraspecific populations occur.

Lateral line counts will usually serve to distinguish individual brown trout from individual cutthroats and steelheads.

Counts made at higher levels do not provide a sound general basis for the specific identification of any of these three species, though clear distinctions may occur between certain populations.

Certain advantages are claimed for a counting method involving the enumeration of oblique scale rows corresponding to a given number of lateral line scales but not involving the posterior part of the body.

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SAMPLING METHODS IN POPULATION STUDIES OF THE
EUROPEAN SPRUCE SAWFLY, *GILPINIA HERCYNIAE*
(HARTIG), IN EASTERN CANADA

By M. L. PREBBLE¹

Presented by J. M. SWAINE, F.R.S.C.

INTRODUCTION

ESTABLISHMENT of a reliable sampling method is generally recognized as a first prerequisite to a serious study of insect populations. The method appropriate to a particular problem will depend upon the habits of the insect species, the type of environment and the nature of the distribution of the insect throughout the environment, but must meet the requirement of practicability under actual field conditions. In the case of field-crop insects, recent work (9-15, 17, 18, 20, 21) has been concerned with investigation of the variability of insect populations within and between fields, with various sampling techniques and the reliability of the data. The forest environment differs from that encountered in field-crop studies in such respects as the varying composition and density of the stand, size, shape, and age of the trees, and the character of the ground, and these factors might be expected to increase the variability of the insect populations within and between forest stands. Methods of sampling forest insect populations which have been used (19), differ widely according to the habits of the species. Not infrequently, however, population data for forest insects have been presented without appraisal of their statistical reliability, or of the adequacy of the sampling method to provide data having a reasonable degree of precision.

The serious outbreak of the European spruce sawfly in Eastern Canada raised many practical questions, such as the probable population trend, the importance of natural control factors, and particularly, the relative success of the several introduced parasitic species, which had to be answered on the basis of quantitative field studies. The development of a technique for sampling populations of the European spruce sawfly, by which the desired information might be obtained, was therefore one of the important objectives in the investigations carried out by the Division of Entomology, Dominion

¹Contribution No. 2241. Division of Entomology, Science Service, Department of Agriculture, Ottawa.

Department of Agriculture, on this insect in Eastern Canada. Preliminary studies of sampling methods were started in the valley of Berry Mountain Brook in the central part of the Gaspé Peninsula in 1931, and more intensive sampling was carried on in the same and in a neighbouring valley from 1932 to 1940. As the sawfly outbreak extended to south-central New Brunswick, the investigations of sampling methods were enlarged to take in the newly infested territory, and intensive counts have been made in this region from 1934 to the present time. From the very extensive statistics gathered during the past decade, certain data have been used in the annual reports on the sawfly outbreak (1-3, 5-8), and in special studies relating to natural control and survival (23, 24), and to the need of salvage of spruce in relation to sawfly population density (4).

The present paper, based on field data collected over the interval 1932-40, reports on the methods of sampling and analysis of the data. Early attempts at studying larval populations in the tree crowns led to the conclusion that the various methods, while useful for observations on seasonal development and gross changes in numbers, were unsuitable as a source of reasonably precise data. Studies of the larvae falling from the trees on to specially designed trays were also carried out (22), providing useful information on seasonal trends. Intensive studies of larval populations require daily or very frequent observations throughout the entire period of development, and this, of course, severely limits the number of points at which the studies can be carried out. Moreover, they have the serious deficiency that the data so gathered provide no information on the number of individuals actually spinning cocoons, on the importance of control factors affecting the cocoons, or on the reserve of sawflies in diapause, which is of great importance in the epidemiology of this species (24). The cocoon, however, which is spun in the moss and debris of the forest floor, is eminently suitable for intensive studies, because it is durable even after the occupant has emerged or been killed and thus gives evidence of the various control factors operating against the cocoons, and because of the regulation which can be exercised over the forest floor as a sampling universe. The present paper is concerned solely with sampling methods applicable to the cocoon. Many plots were studied in the Gaspé Peninsula and at the Parke Forest Reserve in the Province of Quebec, and at numerous points in south-central New Brunswick, over the period 1932-40, to supply information on sawfly cocoon populations. Since it is impossible and unnecessary to refer to more than a few plots here, the writer has selected eight plots,

here given arbitrary numbers starting at one, which serve to illustrate the separate phases in the development of the sampling technique.

METHOD I. SAMPLING THE "WHOLE FOREST"

The first method of sampling was designed to provide quantitative data applicable to the "whole forest." Rectangular plots 100 feet square were laid out in a number of representative stands in different forest types in central Gaspé in 1932. The location of 25 sample units, each 2 feet square, was determined arbitrarily by the intersection points of cross strings stretched at 20-foot intervals between opposite sides of each plot. The second series of sample units in each plot was located by shifting the cross strings 10 feet.

Each sample unit was laid out on the ground by using a wooden frame, and all moss and debris lying within the frame were reduced to shreds, taking a handful or so at a time on to a cloth apron held on the lap of the worker. It was most economical of time to take off the moss in successive layers, and so to continue until no more cocoons could be found. This usually meant careful examination down to the upper layers of the mineral soil, or a sampling depth range from 2 or 3 inches in stands with little or only shallow moss to as many as 8 or 9 inches in stands with deep moss. Cutting around the edges of the frame with a long-bladed knife tended to reduce errors due to border effect.

All cocoons, kept separate for each quadrat, were sorted into the different categories by one worker, so far as this was possible, in order that variability due to personal judgment might be reduced to a minimum.

Descriptions of one plot in each of the three important spruce types of central Gaspé are shown in Table I, and 1932 population data for these plots appear in Table II. The spruce volume was about equal on plots I and II, at 23 cords to the acre, but approximately one-third less on plot III. However, a few large trees made up the bulk of the spruce volume on plot I, and balsam fir, which is not fed upon by the spruce sawfly, was an important element in the stand, and so it was to be expected that variability in cocoon density would be greater in plot I than in plot II, which was essentially a pure black spruce stand. While plot III was also almost pure black spruce, increased variability in cocoon density was encouraged by the more open stand and by the fact that the sawfly infestation in the area represented by the plot was comparatively recent and still light.

The calculated mean differences in cocoon population per sample

quadrate for the three plots, representing the change between June and October, are shown in Table III. Only four of the eighteen differences are statistically significant, and of these four, only the two differences pertaining to sound cocoons can be accepted as reasonable. The other two differences are negative, implying a reduction in the cocoons killed by insects and in the total cocoons in the ground, during the summer, while in fact no reduction is possible in so short a period owing to the durability of the cocoon. Part of the apparent reduction in the number of cocoons in several of the categories other than sound cocoons may be attributed to sampling fluctuations, but the failure to recover equal proportions of the cocoons at the two sampling periods must have been an important factor. The October samples were taken under trying conditions, the moss being very cold, occasionally partly frozen. From data subsequently gathered on the proficiency of workers there can be little doubt that many cocoons were missed in the October samples. The June samples, however, were taken under favourable conditions and are satisfactory for appraisal of the sampling method.

One criterion of the practicability of the method is the estimated number of sample units required to define the mean within acceptable limits. It is presumed that the sample mean does not differ significantly from the population mean, but since chance fluctuations alone may account for deviations in the mean equal to about twice the standard error, it follows that the population mean may lie anywhere within the limits of twice the standard error from the sample mean. Refinement in the estimate of the population mean therefore depends upon the magnitude of the standard error determined in the sample, and this varies inversely as the root of the sample size. If it be desired that the estimate of the mean deviate not more than 5 per cent from the population mean, then the standard error must not exceed $2\frac{1}{2}$ per cent of the mean. Accepting the standard deviation provided by the sample, and setting standard error at $2\frac{1}{2}$ per cent of the mean, the required sample size is calculated as follows:

$$S.E. \bar{x} = \frac{S.D. x}{\sqrt{N}} \dots\dots\dots (1)$$

$$\text{whence } N = \left[\frac{S.D. x}{S.E. \bar{x}} \right]^2 \dots\dots\dots (2)$$

The values of N based on the June samples for the sound, emerged and total cocoons, in which categories variability was least, are shown in Table IV. In each category and in each plot, even plot II which

TABLE I
DESCRIPTION OF THE FOREST REPRESENTED BY THREE SAMPLE PLOTS USED IN THE FIRST METHOD OF
SAMPLING, CENTRAL GASPÉ, 1932

Plot no.	Forest type	Age of stand	Drainage	Dominant ground cover	Stand table (trees per acre by diam.)						Vol. per acre (cords)
					4-5"	6-7"	8-9"	10-11"	12-13"	14-15"	
I	white spruce flat	150-200 years	poor	shallow moss, bunchberry, twin flower, wood sorrel, snowberry	wh. sp. 34	53	52	26	18	9	23 6
					bl. sp. 9	22	9	4	.	.	3 3
					balsam 91	65	30	9	4	.	11 3
II	black spruce slope	200-250 years	good	deep moss, snowberry, rock cranberry, labrador tea, lambkill, bunchberry	bl. sp. 253	183	65	4	.	.	23 0
					balsam 4	0 1
III	black spruce flat	210-220 years	medium	moss, depth medium, snowberry, labrador tea, lambkill, bunchberry	bl. sp. 166	117	48	8	.	.	15 9
					balsam 17	0 4

TABLE II

POPULATION DATA FOR THREE PLOTS IN CENTRAL GASPÉ, JUNE AND OCTOBER, 1932

Each sample consisted of 25 quadrates, 2' X 2', laid out in the "whole forest."

Plot no.	Sampling period		Sound	Emerged	Killed by mammals	Killed by insects	Dead	Total
I	June..	Mean, \bar{x} ...	26.08	6 12	17.08	1.24	4 40	54.92
		<i>S.D.x</i> ...	26.58	6 94	19 92	1 83	5 85	55 69
		coeff. var. .	102%	114%	117%	148%	133%	102%
		<i>S.E.x</i>	5 32	1 39	3 98	0 37	1.17	11.14
	October	Mean, \bar{x} ..	11.52	3 68	15.28	1 60	8.60	40 68
		<i>S.D.x</i> ..	10 87	4 06	21 79	2 28	11 99	36 18
		coeff. var. .	94%	110%	143%	143%	140%	89%
		<i>S.E.x</i>	2.17	0 81	4.36	0 46	2 40	7 24
	June ..	\bar{x}	57.48	46.08	66.28	14 04	5 92	189.80
		<i>S.D.x</i> ..	35.62	29 24	54.24	15 26	8 06	111 39
		coeff. var. .	62%	63%	82%	109%	136%	59%
		<i>S.E.x</i>	7.12	5 85	10.85	3 05	1 61	22 28
II	October	\bar{x}	35.92	35 08	52.36	7 36	3 24	133 96
		<i>S.D.x</i> ..	16.90	20 70	33.17	6 26	4 03	70.84
		coeff. var. .	47%	59%	63%	85%	124%	53%
		<i>S.E.x</i>	3 38	4 14	6 63	1.25	0 81	14 17
III	June ..	\bar{x}	11.04	4.28	10.16	0 28	0 48	26.24
		<i>S.D.x</i> ..	9 69	3 15	10 00	0 46	0 77	17 31
		coeff. var. .	88%	74%	98%	164%	160%	66%
		<i>S.E.x</i>	1.94	0 63	2 00	0 09	0.15	3 46
	October	\bar{x}	11.56	6.08	15.72	0.76	0.44	34 56
		<i>S.D.x</i> ..	10 77	4.30	19 03	1 64	0.65	30.46
		coeff. var. .	93%	71%	121%	216%	148%	88%
		<i>S.E.x</i>	2 15	0 86	3 81	0.33	0.13	6.09

$$S.D.x = \sqrt{\frac{Sx^2 - \frac{(Sx)^2}{N}}{N-1}} \quad \text{coefficient variability} = 100 \frac{S.D.x}{\bar{x}}$$

$$S.E.\bar{x} = \frac{S.D.x}{\sqrt{N}}$$

was almost a pure stand of black spruce trees of fairly uniform size and distribution, the estimated number of sample units required for precision of the mean within 5 per cent was entirely beyond practical limits. If the acceptable precision be dropped to 10 per cent, the

number of sample units (one-quarter of the values shown in Table IV) would still exceed the capacity of a field crew of four to six men, permitted, let us say, no more than a week for a single plot.

In an attempt to find a more suitable sampling technique two possibilities were considered: (1), to use the same universe, viz., the whole spruce forest, but to reduce the sample unit size below the $2' \times 2'$ quadrat employed in 1932, so that more units might be secured; (2), to re-define the sampling universe, with or without change in the size of unit. However, the first possibility was rejected

TABLE III

CALCULATED MEAN DIFFERENCES IN COCOON POPULATION PER UNIT AREA ($2' \times 2'$) FOR THREE PLOTS STUDIED IN JUNE AND OCTOBER, 1932 (Table II)
October data are in each instance taken as the minuend.

Plot no.		Sound	Emerged	Killed by mammals	Killed by insects	Dead	Total
I	Mean difference	-14 56	-2 44	-1.80	0 36	4 20	-14.24
	S.E. mean diff. .	5 74	1 61	5 90	0 59	2 67	13 28
	Significance*	strong	none	none	none	none	none
II	Mean difference	-21 56	-11 00	-13 92	-6 68	-2 68	-55.84
	S.E. mean diff. .	7.88	7 17	12 72	3 30	1 80	26.40
	Significance	strong	none	none	(good)†	none	(good)†
III	Mean difference	0 52	1.80	5 56	0 48	-0 04	8.32
	S.E. mean diff. .	2 90	1 07	4 31	0 34	0 20	7 00
	Significance	none	none	none	none	none	none

*For 48 degrees of freedom, required " t " $\left(\frac{\text{Mean diff.}}{\text{S.E.}} \right)$ at 0.05 level, is 2.01; at 0.01 level, 2.68.

†Significant negative differences in all categories except sound cocoons are indicative of systematic error in sampling. See text.

TABLE IV

NUMBER OF SAMPLE UNITS REQUIRED FOR DEFINITION OF MEANS WITHIN 5 PER CENT OF POPULATION MEANS IN DIFFERENT CATEGORIES OF COCOONS
Based on sample data, June, 1932 (Table II).

Plot no.	Sound cocoons	Emerged cocoons	Total cocoons
I	1,660	2,058	1,645
II	614	644	551
III	1,233	867	696

without field tests, since a reduction in the sample unit size while retaining the broad universe would inevitably increase still further variability in cocoon density, in relation to such factors as proximity to spruce trees, the suitability of the ground cover, etc. Therefore, improvement of the sampling method was sought through re-definition of the sampling universe.

METHOD II. SAMPLING A RESTRICTED UNIVERSE

It was known from field observations that fallen, mature larvae ready for spinning tend to burrow into the moss or duff with little migration on the forest floor. To determine the nature of the distribution of cocoons on the floor, counts of the cocoons in one-square-foot quadrates along radii starting at the trunk were made under a number of more or less isolated spruce trees in the stand. Results of counts made in Gaspé are summarized in Table V. In these trees there was

TABLE V

STUDY OF THE DISTRIBUTION OF SAWFLY COCOONS IN THE FOREST FLOOR UNDER THE TREE CROWNS, SHOWING THE TOTAL COCOONS IN SUCCESSIVE ONE-SQUARE-FOOT QUADRATES STARTING AT THE TRUNK AND EXTENDING TO OR BEYOND THE PROJECTION OF THE CROWN MARGIN

The italicized quadrate on each radius is the last one to lie within the projection of the crown margin. Central Gaspé, 1933.

Tree no.	Radius	Successive quadrates					
		1	2	3	4	5	6
1, bl. sp. slope	N	60	43	107	69	23	..
	E	93	56	94	33	12	26
	S	63	58	49	188	47	..
	W	38	52	155	94	63	75
	Ave.	63.5	52.2	101.2	96.0	36.2	(50.5)
2, bl. sp. slope	N	75	27	19	4	4	..
	E	76	39	36	12	7	..
	S	78	43	54	43	26	..
	W	50	43	102	41	12	.
	Ave.	69.7	38.0	52.7	25.0	12.2	..
3, wh. sp. flat	NE	12	20	30	35	48	31
	S	21	23	27	26	63	16
	NW	25	18	26	22	17	..
	Ave.	19.3	20.3	27.7	27.7	42.7	23.5

a reduction in numbers of cocoons in quadrates lying beyond the vertical projection of the crown margin, but no definite trend within the area so circumscribed. Small mammals frequently accumulate considerable hoards of cocoons in hollows at the junction of large roots with the trunk, and this tendency, while not strikingly illustrated in the distributions shown, was reflected in the greater proportions of chewed cocoons in the innermost quadrates for each tree; e.g. in tree no. 1, 75 per cent compared with an average of 63 per cent for the entire tree, in tree no. 2, 58 per cent as against 54 per cent, and in tree no. 3, 65 per cent as against 33 per cent. Elimination of the quadrates lying outside the crown margin reduced variability in trees no. 1 and no. 2; but elimination of the innermost quadrates as well was not consistently followed by a further reduction in variability. In the case of tree no. 3, variability was about the same for the entire sample, for the restricted sample omitting the outer quadrates, and for the restricted sample with both outer and innermost quadrates omitted. While analysis of the 1933 data provided no argument for the exclusion from the sampling universe of the innermost band around the tree trunk, in practice this region was eliminated because of irregularities due to root swelling.

Subsequent distribution studies carried out in south-central New Brunswick in 1938 led to essentially the same conclusions as those reached in the Gaspé studies. There was a marked reduction in cocoon density in quadrates lying beyond the crown margin, and also there was a strong reduction in the inner bands around the trunk.

The sampling universe adopted in the second method included the moss and loose duff lying within the limits of about one foot from the trunk to the vertical projection of the crown margin, of dominant and codominant spruce trees with good crown development and typical of the stand in degree of defoliation. Very small trees and occasional "immune" trees were excluded from the sampling series, since they would inevitably increase variability, which was contrary to the purpose of universe restriction. The trees for sampling and the location of quadrates under each tree were selected by one worker, who drove numbered stakes at the centre of two or more comparable areas of ground cover under each tree. The sample unit size was held at $2' \times 2'$.

Data relating to three plots in central Gaspé are considered here. Plot IV was laid out in the same stand as plot II, the description summarized in Table I applying equally to plot IV; sample units were taken under 133 black spruce trees both in June and October of 1933. Plot V was laid out in another black spruce slope stand in the

same valley, and we shall consider samples taken under 56 black spruce trees in June, 1934, and one year later. Plot VI occurred in a third black spruce slope stand in a neighbouring valley, and samples were taken in June both in 1935 and 1936. The sample data are summarized in Table VI, and the mean differences between successive samples for the same plot are analysed in Table VII.

Several values of the standard error of the mean difference are shown in Table VII. The first, the uncorrected *S.E.*, is calculated from the equation

$$S.E. [\bar{x} - \bar{y}] = \sqrt{[S.E. \bar{x}]^2 + [S.E. \bar{y}]^2} \dots\dots\dots (3)$$

TABLE VI

POPULATION DATA FOR THREE PLOTS IN CENTRAL GASPÉ, 1933-6

The samples consisted of a number of quadrates, 2' X 2', laid out in the restricted universe under the crowns of spruce trees.

Plot no.	Sampling period		Sound	Emerged	Killed by mammals	Killed by insects	Dead	Total
IV	June, 1933 N = 133	Mean, \bar{x} ...	34.68	42.35	70.15	11.20	7.22	165.60
		<i>S.D.x</i> ...	13.02	22.07	51.52	10.48	9.74	65.83
		coeff. var.	38%	52%	73%	94%	135%	40%
		<i>S.E.x</i> ...	1.13	1.91	4.47	0.91	0.84	5.71
	October, 1933 N = 133	\bar{x}	59.44	45.38	61.19	9.13	7.32	182.46
		<i>S.D.x</i> ...	36.04	20.13	32.15	10.67	10.33	62.95
		coeff. var.	61%	44%	53%	117%	141%	35%
		<i>S.E.x</i>	3.12	1.75	2.79	0.93	0.90	5.46
V	June, 1934 N = 56	\bar{x}	90.45	39.09	80.89	9.48	7.89	227.80
		<i>S.D.x</i>	38.71	22.29	46.51	7.23	6.52	79.32
		coeff. var.	43%	57%	58%	76%	83%	35%
		<i>S.E.x</i>	5.17	2.98	6.21	0.97	0.87	10.60
	June, 1935 N = 56	\bar{x}	43.86	47.71	119.71	9.93	7.46	228.67
		<i>S.D.x</i> ...	20.20	21.73	84.54	9.23	6.91	99.35
		coeff. var.	46%	46%	71%	93%	93%	43%
		<i>S.E.x</i> ...	2.70	2.90	11.29	1.23	0.92	13.30
VI	June, 1935 N = 50	\bar{x}	70.26	51.38	83.12	20.88	16.10	241.74
		<i>S.D.x</i>	28.25	22.86	51.34	15.19	11.57	88.67
		coeff. var.	40%	45%	65%	73%	72%	37%
		<i>S.E.x</i> ...	3.99	3.23	7.68	2.15	1.64	12.54
	June, 1936 N = 50	\bar{x}	54.08	61.46	91.06	19.50	20.88	246.98
		<i>S.D.x</i>	19.11	28.44	58.53	11.82	15.48	94.19
		coeff. var.	35%	46%	64%	61%	74%	38%
		<i>S.E.x</i>	2.70	4.02	8.28	1.67	2.19	13.32

TABLE VII

CALCULATED MEAN DIFFERENCES IN COCOON POPULATION PER UNIT AREA ($2' \times 2'$)
OF THE RESTRICTED UNIVERSE

Data in Table VI. The later sample in each plot taken as the minuend.

Plot no.		Sound	Emerged	Killed by mammals	Killed by insects	Dead	Total
IV	Mean difference .	-24 76	3 03	- 8 96	-2 07	0.10	16.86
	<i>S.E.m.d.</i> uncorrected.	3 32	2 59	5 27	1 30	1 23	7 90
	" <i>t</i> " and significance..	7.45, strong	1.17, none	1 70, none	1 59, none	0 08, none	2 14, good
	correlation coeff., <i>r</i> .	(-0.109)	+0 394	+0 266	+0.365	+0 628	+0 219
	<i>S.E.m.d.</i> corrected . .	.	2 02	4 59	1 04	0 75	6 98
	" <i>t</i> " and significance .	.	1 50, none	1 95, marginal	1 99, marginal	0 13, none	2 41, good
	"Ideal" <i>S.E.m.d.</i> . .	1 72	1 55	2 33	0 36	0 26	6 16
V	Mean difference	-46 59	8 62	38 82	0 45	-0 43	0 87
	<i>S.E.m.d.</i> uncorrected	5 83	4 15	12 88	1 56	1 27	17.01
	" <i>t</i> " and significance.	8 00, strong	2 08, good	3 01, good	0 29, none	0 34, none	0 05, none
	<i>r</i> .	+0 498	+0 488	(+0.164)	+0 518	+0 328	+0 424
	<i>S.E.m.d.</i> corrected	4 48	2 96	.	0 91	1 04	13.13
	" <i>t</i> " and significance . .	10.39, strong	2 91, good	.	0 49, none	0 41, none	0 07, none
	"Ideal" <i>S.E.m.d.</i> . .	2.51	1 54	3 61	0 34	0 27	8 08
VI	Mean difference .	-16 18	10 08	7 94	-1 38	4.78	5 24
	<i>S.E.m.d.</i> uncorrected	4 82	5 16	11 29	2 72	2.74	18 29
	" <i>t</i> " and significance	3 35, good	1.95, marginal	0 70, none	0 51, none	1 75, none	0.29, none
	<i>r</i> . .	(+0.189)	+0 313	+0 303	(+0.162)	+0 443	+0 343
	<i>S.E.m.d.</i> corrected	.	4 30	9 43	.	2 08	14 84
	" <i>t</i> " and significance	.	2 34, good	0 84, none	.	2 30, good	0 35, none
	"Ideal" <i>S.E.m.d.</i> . .	2.22	2 00	3 09	0 72	0 66	8 64

which implies the absence of correlation between the values of x and y . The first determination of the statistic "*t*" and conclusion regarding the significance of the mean difference are based on the uncorrected standard error.

The second value of the standard error of the mean difference has been corrected for correlation between the individual values of x and y

(numbers of cocoons taken in the quadrates under individual trees at different sampling periods), according to the basic equation

$$S.E. [\bar{x} - \bar{y}] = \sqrt{[S.E.\bar{x}]^2 + [S.E.\bar{y}]^2 - 2r[S.E.\bar{x}][S.E.\bar{y}]} \dots\dots(4)$$

in such instances where the correlation coefficient proved significant. The significance of r was determined from the relation (see 16, p. 72)

$$t = \frac{r \sqrt{n}}{\sqrt{1-r^2}} \dots\dots\dots(5)$$

where n , the number of degrees of freedom, is two less than the number of paired variates. The second conclusion regarding the significance of the mean difference in Table VII is based on the corrected standard error.

The third or "ideal" value of the standard error of the mean difference is a fictitious value calculated for comparative purposes as described later. It is based on the assumption that the individual standard errors of \bar{x} and \bar{y} were $2\frac{1}{2}$ per cent of the respective means, and that there was no correlation between x and y . In other words, the "ideal" standard error of the mean difference corresponds to the value that would have been obtained had the separate sample means been defined within limits of 5 per cent and had there been no correlation between the numbers of cocoons derived from the same trees at different times, or had the samples been collected in such a way that correlation could not have been taken into consideration.

The method of sampling in a restricted universe resulted in a consistent reduction in variability, as may be seen by comparison of the coefficients of variability in Tables II and VI. The reduction in variability of total cocoons per sample unit in the black spruce slope type, as compared with the first method of sampling, was in the approximate relation of 38 to 56. The estimated number of sample units required for precision of the means within 5 per cent (Table VIII), though still larger than could be handled under normally prevailing conditions, is much reduced from the requirements of the first method. Thus the general average for Table VIII, which is 297, is comparable with 603, which is the average number of sample units required in the same forest type by the first method of sampling (Table IV, plot II).

In the analysis of the mean differences (Table VII), it will be noted that of eighteen tests of significance based on the uncorrected standard errors, six differences were significant and one marginal.

Instances of apparent but actually impossible reductions in categories other than sound cocoons were reduced in frequency though not entirely eliminated.

After the reduction in variability of cocoon density, a further advantage of the second method of sampling relates to the correlation between the sample units from the same trees at different times. This, as already indicated, has been used in the calculation of the corrected standard errors. The minimum value of the correlation coefficient for significance ($P = 0.05$) varied somewhat between the different plots, in relation to $N - 2$, being 0.170 for plot IV, 0.263 for plot V, and 0.279 for plot VI. Fourteen of the eighteen coefficients

TABLE VIII

NUMBER OF SAMPLE UNITS REQUIRED FOR DEFINITION OF MEANS WITHIN 5 PER CENT OF POPULATION MEANS IN DIFFERENT CATEGORIES OF COCOONS

Based on sample data from the restricted universe (Table VI).

Plot no.	Period	Sound cocoons	Emerged cocoons	Total cocoons
IV	June, 1933	225	434	253
V	June, 1934	293	520	194
	June, 1935	340	332	302
VI	June, 1935	259	317	215
	June, 1936	200	343	232
	Average	263	389	239

Over-all average, 297.

were significant, and the degree of reduction in the standard errors corrected for correlation varied from 12 to 42 per cent, averaging about 23 per cent, of the corresponding uncorrected values. The significance of one mean difference (emerged, plot VI), formerly doubtful, was established on the basis of the corrected standard error. Two other differences considered not significant in the first test, were placed near the 0.05 level in the second test (plot IV, killed by insects and by mammals), though it must be admitted that the near-significance indicates failure to recover cocoons in the second sample (October) rather than a reduction in numbers, as suggested by the negative differences.

However, the corrected standard errors were in every instance

greater than the fictitious "ideal" standard errors, by from 13 to 285 per cent in individual cases, and averaging about 120 per cent. Despite improvement in reduced variability and in allowance for correlation, it was obvious that the second sampling method could not be depended upon to measure accurately any but comparatively large changes in cocoon density. Possibility of further improvement lay in the redistribution of individual sample units in order to provide a more accurate estimate of the cocoon density under each tree in the series at each sampling period.

METHOD III. DISTRIBUTION OF SUB-SAMPLES WITHIN THE RESTRICTED UNIVERSE

In considering improvement in the method of sampling, it was desired to retain the area to be sampled under each tree at each sampling period at 4 square feet, in order that the data might be comparable to those obtained by the second method. Convenience in size led to the adoption of one-square-foot sub-samples, and this permitted the distribution of four such sub-samples within the sampling area under each tree. The sum of the four sub-samples for each tree was to be regarded as the sample unit in the series of counts.

Division of the sample unit into a number of parts required that particular precautions be taken to avoid the selection of choicest locations for the early samples. Therefore, the areas to be examined at each sampling period were rigorously determined at the time the plots were laid out. The procedure was as follows:

(1) First the investigator became familiar with the stand to be studied by means of a general survey, in which impressions were formed regarding the size of the trees and the degree of defoliation, and the nature of the ground cover through the stand. Then a series of dominant and codominant trees of good crown development and overlying sufficient favourable ground cover for the sampling, was selected throughout the stand. When the plans for a particular stand envisaged annual samples for four years, 50 trees were selected and each tree was included in each annual sample. If the plans envisaged annual samples for eight years, 100 trees were selected, the odd-numbered ones being used for the first four years, and the even-numbered ones for the subsequent samples.

(2) The suitable ground cover under each selected tree was apportioned at once into four areas, usually on different sides of the trunk, and into the centre of each such area a square wooden stake was driven. The stakes were designated *A*, *B*, *C*, or *D*, and the sides

of each stake were numbered in sequence from 1 to 4. Preparatory to driving the stake, it was tossed and caught at random by the operator, so that in driving, sometimes side no. 1 would be adjacent to the trunk, sometimes side no. 2, and so on, but entirely in a random fashion.

(3) In the actual sampling, one-square-foot frames were set down at predetermined corners of each stake, and the cocoons obtained in the area so delimited were classified and recorded separately. The origin of each sub-sample was completely identified by the tree number, stake letter, and stake side number. For convenience, the

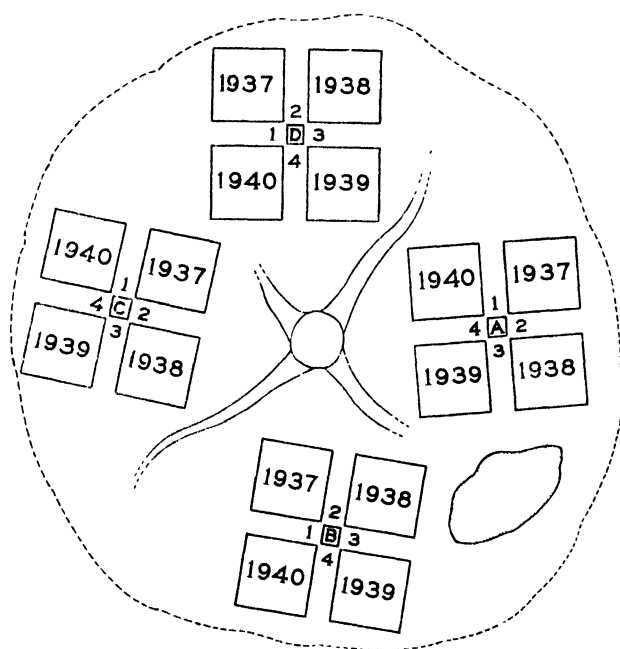


FIGURE 1.—Diagram illustrating the method of laying out the sub-samples within the area of crown projection under each tree in the sample series. Roots, rocks, and other unfavourable areas on the ground were avoided. The numbers in the one-square-foot quadrates indicate the year in which each sub-sample was taken.

first sub-samples were taken diagonal to the 1-2 corner of each stake, the second at the 2-3 corner, etc., and a control strip equal at least to the width of the stake separated successive sub-samples at each stake (Fig. 1).

The third sampling method was tried out intensively in two plots. Plot VII extended over several acres in the black spruce slope type

in central Gaspé, in the same stand which had been represented by plot VI in the 1935 and 1936 studies. The number of trees per acre is summarized in the synopsis.

	4-5"	6-7"	8-9"	10-11"	Volume (cords)
Black Spruce..	156	146	60	11	19.7
Balsam	32	14	1	..	1.7
White birch	2	1	0.1

The effects of sawfly defoliation had already killed about 28 per cent of the black spruce trees. The dead trees were distributed almost proportionately throughout the different diameter classes. In laying out the plot, however, only living trees were included in the series tagged and staked for population studies in 1937 and later. The moss carpet in plot VII was uniformly deep.

Plot VIII, with an area of 7.2 acres, was established in a black spruce flat type on the Acadia Forest Experiment Station, Sunbury County, New Brunswick. The stand composition was more varied than the black spruce stands of Gaspé, as shown in the accompanying synopsis.

	4-5"	6-7"	8-9"	10-11"	12-13"	Volume
Black spruce.	110	91	32	5	0.3	9.3
White spruce.	1	1	0.5	0.1	...	0.1
Balsam.	3	1	0.3	0.1	...	0.2
White pine.	0.7	0.4	0.4	1	0.9	0.4
Larch.	1.2	1.5	0.1
Poplar.	7	2	0.2
Birch.	31	6	0.7	1.3
Red Maple.	6	1.5	0.2

The black spruce trees were shorter, with wider crowns and a relatively greater crown volume than the Gaspé black spruce. The age of the stand was about fifty years, and there was no mortality as a result of sawfly defoliation. The moss carpet was shallow and incomplete, loose litter and duff occupying much of the forest floor.

Sample data obtained in 1937 for plot VII are shown in Table IX. The variability in the various categories of cocoons was appreciably less than in the samples obtained by the second method of sampling (Table VI), providing evidence of the increase in precision which might be expected from sub-division of the sample unit taken under each tree.

With the improved technique now at hand, the question of accuracy of cocoon recovery from the moss and humus, which had been left in abeyance during the preliminary stages of the investigations, was now thoroughly investigated. This took the form of a complete check of the 200 sub-samples examined in June, 1937. It seemed at first that it might be possible to apply correction factors to the unchecked counts, to correct for cocoons missed in the original examination, but, anticipating results described in a subsequent section, it may be said here that such a procedure was found to be impracticable. It therefore became standard practice in 1937 to check completely all sample units.

TABLE IX

POPULATION DATA FOR PLOT VII, BLACK SPRUCE SLOPE, CENTRAL GASPÉ, JUNE 1937

Each of the fifty units in the sample consisted of four one-square-foot sub-samples taken under the crown of a dominant or codominant spruce tree.

	Sound	Emerged	Killed by mammals	Killed by insects	Dead	Total
Mean, \bar{x}	32 50	65 00	107.46	16.00	13.66	234.62
S.D.x. . . .	12 14	20 35	56 39	10 17	8 13	67 46
Coeff. var. . . .	37%	31%	52%	64%	60%	29%
S.E. \bar{x} . . .	1 72	2 88	7 98	1 44	1.15	9.54

Series of *double* checks were carried out in the field to establish the absolute accuracy of checked sample data. The degree of accuracy varied within narrow limits for sub-samples which had been examined and checked by different combinations of workers, and also, between the different categories of cocoons. The average values for plots VII and VIII are shown in the synopsis.

PERCENTAGES OF THE ABSOLUTE NUMBER OF COCOONS TAKEN IN ORIGINAL AND FIRST CHECK EXAMINATIONS, AS DETERMINED BY A NUMBER OF DOUBLE CHECKS

Sub-samples for double checking taken at random from the sample series, without fore-warning to the first checker.

	Sound	Emerged	Killed by mammals	Killed by insects	Dead	Total
Plot VII						
23 sub-samples	99.2	95.8	95.8	96.6	86.5	95.6
Plot VIII						
20 sub-samples	100	99.0	96.5	98.3	100*	98.3

*Based on only 16 dead cocoons in the 20 sub-samples.

The error in sound cocoons was least, that relating to dead cocoons, which are often very dark, broken, and deeply buried, was greatest. In this connection the data for dead cocoons on plot VIII should be discounted since so few cocoons were involved. On the whole, the error for plot VII was less than 5 per cent, that for plot VIII from 1 to 3 per cent. This departure from absolute accuracy in recovery of cocoons is inherent in all sample data of these two plots from 1937 onwards.

Sample data for the two plots are shown in Table X, and analysis of mean differences in Table XI. As anticipated from the original unchecked data of plot VII, 1937, variability in the checked data of 1937-40 for this plot was much reduced from that associated with data obtained by the second sampling method, though in the case of sound cocoons there was an increasing variability with declining numbers in later years. The estimated number of sample units required for precision of the means within 5 per cent (Table XII) averaged 196 as contrasted with 297 by the second method. The correlation between successive units from the same source was also considerably higher in plot VII than in plots IV, V, and VI, all coefficients proving significant. Correspondingly, there was an increased reduction in the standard errors of the mean differences corrected for correlation; this varied from 20 to 50 per cent, with an average of about 36 per cent, below the uncorrected standard errors. Two mean differences for plot VII, whose significance was unproved on the basis of uncorrected standard errors, were proved significant in the tests with corrected errors. Finally, four of the corrected errors for plot VII were less than the theoretical "ideal" standard errors. This represents the closest approach to the attainment of the degree of precision which had been set as an ideal in 1932.

The data for plot VIII illustrate the same general tendencies as those noted in the preceding paragraph. Variability was somewhat greater than in plot VII, correlation not so high though in every case significant; the reduction in standard errors of the mean differences following correction for correlation was of a lower order, ranging from 13 to 49 per cent, and averaging about 23 per cent. One difference of unproved significance on the basis of the first test, was proved significant in the second test. The corrected standard errors were all in excess of the corresponding "ideal" standard errors, but the comparatively great change in sample means in relation to absolute density was a factor tending to give significance to the data for this plot.

TABLE X

POPULATION DATA FOR PLOT VII, BLACK SPRUCE SLOPE, CENTRAL GASPÉ, 1937-40,
AND FOR PLOT VIII, BLACK SPRUCE FLAT, SUNBURY COUNTY, N.B., 1938-40

Each of the fifty units in each sample consisted of four one-square-foot sub-samples taken under the crown of a dominant or codominant spruce tree, and each sub-sample was completely checked after the original count.

Plot no.	Sampling period		Sound	Emerged	Killed by mammals	Killed by insects	Dead	Total
VII	June, 1937	Mean, \bar{x}	35 68	88 68	135 70	20 82	19.88	300.76
		<i>S.D.x</i>	12 87	24 72	68 36	12 99	11 07	77 08
		coeff. var	36%	28%	50%	62%	56%	26%
		<i>S.E.x</i>	1 82	3 49	9 66	1 84	1 56	10 90
	June, 1938	\bar{x}	55 60	109.50	174 92	30 98	23.08	394 08
		<i>S.D.x</i>	24 01	30 63	92 67	15 00	13 67	116 26
		coeff. var	43%	28%	53%	48%	59%	30%
		<i>S.E.x</i>	3 39	4 33	13 11	2 12	1 93	16 44
	June, 1939	\bar{x}	34 38	120 02	183 74	44 96	21 56	404 66
		<i>S.D.x</i>	15 41	29 44	88 94	20.34	13 68	108 86
		coeff. var	45%	24%	48%	45%	63%	27%
		<i>S.E.x</i>	2.18	4 16	12 58	2 87	1 93	15 39
	June, 1940	\bar{x}	21 30	124 60	192 72	50 52	19 06	408 20
		<i>S.D.x</i>	12 33	32 90	102 93	26 65	13 89	133.32
		coeff. var	58%	26%	53%	53%	73%	33%
		<i>S.E.x</i>	1 74	4 65	14 56	3 77	1 96	18 86
VIII	May, 1938	\bar{x}	9.20	7 12	6 24	6 52	0 26	29 34
		<i>S.D.x</i>	5 89	4 19	4 12	3 83	0 56	13.70
		coeff. var	64%	59%	66%	59%	216%	47%
		<i>S.E.x</i>	0 83	0 59	0 58	0 54	0 08	1 94
	May, 1939	\bar{x}	20 96	10 36	9 00	10 86	1 28	52 46
		<i>S.D.x</i>	12 48	5.43	7 64	6 30	1 37	23 62
		coeff. var	59%	52%	85%	58%	107%	45%
		<i>S.E.x</i>	1 76	0 77	1 08	0 89	0 19	3.34
	May, 1940	\bar{x}	5.46	18 10	12 96	18 94	2 74	58 20
		<i>S.D.x</i>	4 59	8 30	5 71	9 90	2 42	24 96
		coeff. var	84%	46%	44%	52%	88%	43%
		<i>S.E.x</i>	0.65	1 17	0 81	1 40	0 34	3 53

ANALYSIS OF INTER- AND INTRA-TREE VARIABILITY

It is clear on theoretical grounds that the total variability in cocoon density in samples taken by the third method derives partly from variability within the area under individual trees, traceable to

irregularities in crown development, in the nature of the ground cover, etc., and partly from variability between the different trees owing to variation in size, to location within the stand, exposure, etc. The purposeful selection of only dominant and codominant trees with suitable ground cover was designed to keep inter-tree variability as low as possible. However, if intra-tree variability were not significantly lower than inter-tree variability, there would be little purpose in taking more than one quadrat under any one tree at each sample

TABLE XI

CALCULATED MEAN DIFFERENCES IN COCOON POPULATION BETWEEN SUCCESSIVE YEARS IN PLOTS VII AND VIII

Data in Table X.

Plot no.		Sound	Emerged	Killed by mammals	Killed by insects	Dead	Total
VII							
1938-	Mean difference .	19 92	20 82	39 22	10.16	3 20	93.32
1937	<i>S.E.m.d.</i> uncorrected	3 85	5 56	16 28	2.81	2 48	19.72
	" <i>t</i> " and significance	5 17, strong	3.74, strong	2.41, good	3.61, strong	1.29, none	4.73, strong
	<i>r</i>	+0.541	+0.628	+0.640	+0.694	+0.698	+0.687
	<i>S.E.m.d.</i> corrected . .	2 85	3 46	10.15	1 58	1.40	11.95
	" <i>t</i> " and significance	6 99, strong	6.01, strong	3.86, strong	6 43, strong	2.28, good	7.80, strong
	"Ideal" <i>S.E.m.d.</i> . .	1.65	3.52	5.53	0 93	0.76	12.39
1939-	Mean difference .	-21.22	10 52	8 82	13 98	-1.52	10.58
1938	<i>S.E.m.d.</i> uncorrected	4.03	6.01	18.18	3.57	2.73	22.52
	" <i>t</i> " and significance	5.26, strong	1.75, none	0.48, none	3.92, strong	0.56, none	0 47, none
	<i>r</i>	+0.801	+0.680	+0.529	+0.683	+0.542	+0.605
	<i>S.E.m.d.</i> corrected	2.09	3.40	12.46	2.11	1.85	14.18
	" <i>t</i> " and significance	10.15, strong	3.09, strong	0.71, none	6 63, strong	0.82, none	0.75, none
	"Ideal" <i>S.E.m.d.</i> . .	1.63	4.06	6.34	1.36	0.79	14.86
1940-	Mean difference	-13.08	4.58	8.98	5.56	-2.50	3 54
1939	<i>S.E.m.d.</i> uncorrected	2.79	6.24	19.24	4.74	2.75	24.34
	" <i>t</i> " and significance	4.68, strong	0.73, none	0 47, none	1.17, none	0.91, none	0.15, none
	<i>r</i>	+0.772	+0.482	+0.361	+0.445	+0.649	+0.405
	<i>S.E.m.d.</i> uncorrected	1 39	4.50	15.43	3.58	1.63	18.90
	" <i>t</i> " and significance	9.40, strong	1.02, none	0.58, none	1.55, none	1.53, none	0.19, none
	"Ideal" <i>S.E.m.d.</i> . .	1 01	4.33	6.66	1.69	0.72	15.09

TABLE XI—*Continued*

Plot no.		Sound	Emerged	Killed by mammals	Killed by insects	Dead	Total
VIII							
1939-	Mean difference . .	11 76	3 24	2 76	4 34	1 02	23.12
1938	<i>S.E.m.d.</i> uncorrected	1 95	0 97	1 23	1 04	0 21	3.86
	" <i>t</i> " and significance	6.03, strong	3.34, strong	2 25, good	4.17, strong	4.85, strong	5.98, strong
	<i>r</i>	+0.448	+0 514	+0 465	+0 288	+0.400	+0.540
	<i>S.E.m.d.</i> corrected .	1 57	0 69	0 96	0 90	0 17	2.82
	" <i>t</i> " and significance	7 49, strong	4 70, strong	2 87, strong	4 82, strong	6 00, strong	8.20, strong
	"Ideal" <i>S.E.m.d.</i> .	0 57	0 31	0 27	0 32	0 03	1.12
1940-							
1939	Mean difference .	-15.50	7 74	3 96	8 08	1 46	5 74
	<i>S.E.m.d.</i> uncorrected	1 88	1 40	1 35	1 66	0 39	4.86
	" <i>t</i> " and significance	8.24, strong	5.53, strong	2 93, strong	4 87, strong	3 74, strong	1.18, none
	<i>r</i>	+0 495	+0.444	+0 422	+0 529	+0 293	+0.739
	<i>S.E.m.d.</i> corrected .	1 55	1 08	1 04	1 20	0 34	2 49
	" <i>t</i> " and significance	10 00, strong	7 16, strong	3 81, strong	6 73, strong	4 29, strong	2 31, good
	"Ideal" <i>S.E.m.d.</i> .	0 54	0 52	0 39	0 55	0 08	1 97

With 48 degrees of freedom, the minimum value of *r* for significance (*P* = 0.05) is 0.279.

period, and in fact it would be quite logical to consider the one-square-foot quadrates as the sample units. These might be taken at convenience within the sampling universe without restriction as to location and number per tree. Freedom from these restrictions, especially if carried to the point where every tree was not necessarily included in every sample, would eliminate the possibility of allowing for correlation in the calculation of difference errors, but this would tend to be compensated for by the increase in the number of sample units. Clearly, both the sampling procedure and the method of analysis of the data rest upon analysis of inter- and intra-tree variability in cocoon density.

The sample data relating to the total number of cocoons taken in the individual sub-samples for plot VII, 1937-40, and for plot VIII, 1938-40, have been subjected to variance analysis, as summarized in Table XIII. The procedure was as follows: For each plot and year, there were 200 sub-samples, at the rate of four per tree for fifty trees. The total sum of squares for the sample was calculated,

$$T.S.S. = \sum_1^{200} x^2 - \frac{(\sum x)^2}{200}. \quad \dots\dots\dots (6)$$

If the four sub-samples under each tree be designated x_a , x_b , x_c , and x_d , the sum of squares for "between trees," reduced to a single sub-sample basis, may be calculated,

$$S.S. \text{ between trees} = \sum_1^{50} \left(x_a + x_b + x_c + x_d \right)^2 - \frac{(\sum x)^2}{200}. \quad \dots\dots\dots (7)$$

TABLE XII

NUMBER OF SAMPLE UNITS (EACH CONSISTING OF 4 ONE-SQUARE-FOOT SUB-SAMPLES)
REQUIRED FOR DEFINITION OF MEANS WITHIN 5 PER CENT OF POPULATION
MEANS IN DIFFERENT CATEGORIES OF COCOONS

Based on data shown in Table X.

Plot no.	Period	Sound cocoons	Emerged cocoons	Total cocoons	
VII	June, 1937	207	124	105	
	June, 1938	298	125	139	
	June, 1939	322	96	116	
	June, 1940	537	112	171	
	Average	341	114	133	Over-all average, 196
VIII	May, 1938	655	556	350	
	May, 1939	567	141	324	
	May, 1940	1,130	337	295	
	Average	784	445	323	Over-all average, 517

The difference between equations 6 and 7, represents the sum of squares for "within trees," as follows,

$$S.S. \text{ within trees} = \sum_1^{200} x^2 - \sum_1^{50} \frac{(x_a + x_b + x_c + x_d)^2}{4}. \quad \dots\dots\dots (8)$$

Equation 8 provides the easier method of determining variance due to intra-tree variability. Exactly the same result is obtained by the more laborious summation of the squared deviations of the four sub-samples under each tree from their mean, for each of the fifty trees.

Of the 199 degrees of freedom available, 49 pertain to the sum of squares resulting from inter-tree variability, 150 to that resulting from intra-tree variability. The variance due to each source is shown in Table XIII. From the table of distribution of F (16, table 96), it is found that an F value of 1.44 corresponds to the 5 per cent point, one of 1.66 to the 1 per cent point. From the data shown for plots VII and VIII, there can be no doubt that even with restriction of the sampling trees to dominants and codominants with good crowns, the inter-tree variability in cocoon density was distinctly greater than intra-tree variability. This proves the logic of a sampling technique based on units relating to the individual tree.

TABLE XIII

VARIANCE ANALYSIS OF INTER- AND INTRA-TREE VARIABILITY IN COCOON DENSITY, PLOTS VII AND VIII

Analysis relates to the total number of cocoons taken in the sample units.

Plot no.	Year	Inter-tree variance	Intra-tree variance	F*
VII	1937	1486 04	917 03	1 62
	1938	3380 06	2087 31	1 62
	1939	2964 11	1566.70	1 89
	1940	4445 08	2261 89	1 97
VIII	1938	46 99	15.99	2.94
	1939	139 66	45 27	3 08
	1940	155 76	69.42	2 24

$$*F = \frac{\text{Larger variance}}{\text{Smaller variance}}$$

It is, however, instructive to determine the effect upon interpretation of the data resulting from consideration of the 200 sub-samples taken under the fifty trees as independent variates. Here, of course, the means are in terms of cocoon density *per square foot*, the standard error is derived as the $\sqrt{200}$ th part of the standard deviation of the population of square foot variates, and correlation between variates in successive samples must be presumed to be zero. Data for plots VII and VIII, 1938-40, are shown in Table XIV and the mean differences analysed in Table XV. While the sample means in Table XIV are just one-quarter of those in Table X, variability of the square foot variates was much greater in each category of cocoons and in each sample series. Considering the data for plot VII, the

status of the mean differences of the square foot variates was not in any instance contrary to the status of the mean differences of the pooled sub-samples, but the precision of the mean differences was in eleven out of twelve comparisons greater in the case of the pooled sub-samples. Thus in plot VII, where correlation was moderately

TABLE XIV

POPULATION DATA FOR PLOTS VII AND VIII, 1938-40, IN TERMS OF COCOON DENSITY PER ONE-SQUARE-FOOT SUB-SAMPLE

Plot no.	Sampling period		Sound	Emerged	Killed by mammals	Killed by insects	Dead	Total
VII	June, 1938	Mean, \bar{x} . . .	13.90	27.38	43.73	7.74	5.77	98.52
		<i>S.D.x</i> . . .	7.88	12.08	42.16	6.18	5.11	49.04
		coeff. var. . .	57%	44%	96%	80%	89%	50%
		<i>S.E.x</i> . . .	0.56	0.85	2.98	0.44	0.36	3.47
	June, 1939	\bar{x}	8.59	30.00	45.94	11.24	5.39	101.16
		<i>S.D.x</i> . . .	5.27	12.25	38.38	7.23	5.02	43.71
		coeff. var. . .	61%	41%	84%	64%	93%	43%
		<i>S.E.x</i>	0.37	0.87	2.71	0.51	0.36	3.09
	June, 1940	\bar{x}	5.32	31.15	48.18	12.63	4.77	102.05
		<i>S.D.x</i> . . .	4.42	13.29	43.67	9.71	5.69	52.91
		coeff. var. . .	83%	43%	91%	77%	119%	52%
		<i>S.E.x</i>	0.31	0.94	3.09	0.69	0.40	3.74
VIII	May, 1938	\bar{x}	2.30	1.78	1.56	1.63	0.07	7.34
		<i>S.D.x</i> . . .	2.27	1.73	1.70	1.57	0.28	4.86
		coeff. var. . .	99%	97%	109%	96%	400%	66%
		<i>S.E.x</i> . . .	0.16	0.12	0.12	0.11	0.02	0.34
	May, 1939	\bar{x}	5.24	2.59	2.25	2.72	0.32	13.12
		<i>S.D.x</i> . . .	4.40	2.19	2.90	2.50	0.62	8.28
		coeff. var. . .	84%	84%	129%	92%	194%	63%
		<i>S.E.x</i>	0.31	0.15	0.21	0.18	0.04	0.59
	May, 1940	\bar{x}	1.37	4.52	3.24	4.73	0.69	14.55
		<i>S.D.x</i> . . .	2.04	3.50	2.67	3.86	1.05	9.52
		coeff. var. . .	149%	77%	82%	82%	152%	65%
		<i>S.E.x</i>	0.14	0.25	0.19	0.27	0.07	0.67

high, the advantage in the calculation of the standard error resulting from a four-fold increase in the number of sample units was more than offset by the increased variability of the smaller units and by failure to take account, in the calculation of difference errors, of correlation.

TABLE XV

CALCULATED MEAN DIFFERENCES IN COCOON POPULATION, PER SQUARE FOOT SUB-SAMPLE, BETWEEN SUCCESSIVE YEARS IN PLOTS VII AND VIII

Data in Table XIV.

Plot no.		Sound	Emerged	Killed by mammals	Killed by insects	Dead	Total
VII							
1939-1938	Mean difference . .	-5.31	2.62	2.21	3.50	-0.38	2.64
	<i>S.E.m.d.</i> uncorrected	0.67	1.22	4.03	0.67	0.51	4.65
	"t" and significance	7.93, strong	2.15, good	0.55, none	5.23, strong	0.75, none	0.57, none
	"t" based on pooled sub-samples (Table XI)	10.15	3.09	0.71	6.63	0.82	0.75
VIII							
1940-1939	Mean difference . .	-3.27	1.15	2.24	1.39	-0.62	0.89
	<i>S.E.m.d.</i> uncorrected	0.48	1.28	4.11	0.86	0.54	4.85
	"t" and significance	6.81, strong	0.90, none	0.54, none	1.62, none	1.15, none	0.18, none
	"t" based on pooled sub-samples (Table XI)	9.10	1.02	0.58	1.55	1.53	0.19
VIII							
1939-1938	Mean difference . .	2.94	0.81	0.69	1.09	0.25	5.78
	<i>S.E.m.d.</i> uncorrected	0.35	0.19	0.24	0.21	0.04	0.68
	"t" and significance	8.40, strong	4.26, strong	2.87, strong	5.21, strong	6.24, strong	8.50, strong
	"t" based on pooled sub-samples (Table XI)	7.49	4.70	2.87	4.82	6.00	8.20
1940-1939	Mean difference . .	-3.87	1.93	0.99	2.01	0.37	1.43
	<i>S.E.m.d.</i> uncorrected	0.34	0.29	0.28	0.32	0.08	0.89
	"t" and significance	11.38, strong	6.65, strong	3.54, strong	6.28, strong	4.63, strong	1.61, none
	"t" based on pooled sub-samples (Table XI)	10.00	7.16	3.81	6.73	4.29	2.31

The comparisons for plot VIII are less striking. Greater precision of the mean differences, as judged by the statistic " t ," attached to the treatment of the 200 sub-samples as independent variates in six of the twelve comparisons. In one instance, t was exactly the same (2.87) by both methods. In the remaining five comparisons, greater precision attached to the treatment of the data as fifty units of four sub-samples each.

However, the comparisons outlined in the two preceding paragraphs are inherently biased in favour of the treatment of the 200 sub-samples as independent variates. This arises from the fact that four sub-samples were taken at each tree, and since intra-tree variability was significantly less than inter-tree variability in these plots, it follows that total variability of the 200 variates was less than would have been the case had the variates been taken at the rate of only one or two per tree. Hence it would appear that the method of pooling sub-samples taken at the rate of several per tree would be preferable for use in stands where intra-tree was less than inter-tree variability, and where there was moderate or high correlation between successive units drawn from the same trees. In the unusual circumstances where these conditions might not obtain (e.g., possibly in plantations with trees of identical size, spacing, exposure, etc.), or in investigations where repeated samples from the same plot were not contemplated, there would be certain advantages in the use of one-square-foot quadrates distributed with less restriction throughout the stand.

NATURE OF THE DISTRIBUTIONS

While the number of sample units taken in each sample by the third sampling method is too small to permit comparison of actual frequencies with those calculated according to the Poisson series or the normal distribution, certain statistics derived from the samples provide a means of determining the general character of the distributions. A characteristic of distributions conforming to the Poisson series is that the standard deviation equals the mean. An inspection of the determined values for the various samples summarized in Table X will indicate great disparity between the mean and standard deviation in thirty-eight out of forty-two instances; in the other four instances the two values differed by 7 to 16 per cent of the mean. The distributions obtained by the third sampling method obviously did not conform to the Poisson series.

For tests as to normality, the statistics g_1 and g_2 , derived from the sums of the second, third, and fourth powers of the deviations of the

individual variates from their mean (16, pp. 28-9), provide a direct measure of the type and degree of abnormality. The variate selected for analysis in this respect was the total number of cocoons in the pooled sub-samples taken under each tree, for the fifty trees constituting a sample. The two statistics were calculated for the samples from plots VII and VIII for the years 1938, 1939, and 1940, with results summarized in the accompanying synopsis. The standard errors of g_1 and g_2 , dependent solely on sample size, are the same for all samples.

	g_1 (skewness)	g_2 (kurtosis)
Standard error	± 0.3366	± 0.6619
Plot VII 1938	+ 1.1565, significant	+ 2.0901, significant
1939	+ 0.6844, significant	+ 0.8880, not significant
1940	+ 1.0065, significant	+ 2.0182, significant
Plot VIII 1938	+ 0.8289, significant	+ 0.3473, not significant
1939	+ 0.4663, not significant	- 0.2941, not significant
1940	+ 0.3493, not significant	- 0.6346, not significant

Positive values of g_1 indicate positive skewness, or an excess of the number of sample units having values less than the mean. Positive values of g_2 indicate leptokurtic or peaked distributions, negative values platykurtic or flat-topped distributions. Four of the six samples provided statistical evidence of positive skewness, and in two instances the distributions were significantly peaked as well. The 1939 and 1940 samples, plot VIII, showed no significant departures from normality.

Rejection of extreme variates in the sample series is predicated on the normality of the distribution. No extreme variates were rejected in the sample series considered here, and in fact could not properly have been rejected, on the basis of the usual criterion, except in the last two samples from plot VIII.

THE NECESSITY OF CHECKING THE SAMPLES

It is axiomatic that the means and other statistical calculations based on the sample data can make no provision for human errors in collecting the data. Though such errors tend to be reduced in the mean differences between successive samples, correction of this kind must be incomplete if the degree of error is variable between persons, times, and the kinds of objects under study. These considerations

led to the decision to check completely all sample units in 1937, when a promising sample technique seemed to be at hand. Complete field checks were carried out on plot VII from 1937 onward, and on plot VIII from its establishment in 1938. The record of cocoons taken in original and in check counts has been kept separately for each category of cocoons, for each sub-sample quadrat, and for each worker. From the extensive data it is possible to deduce certain facts which may be of general interest. In the discussion which follows, the calculations of percentage error are based on the sum of the cocoons taken in the original and in the check examinations. The sums thus determined were found by a series of double checks, as already described, to be sufficiently close to the absolute totals for practical needs.

The personnel participating in the field work consisted entirely of technical or especially trained workers. Casual labour has not been employed, though such labour might be satisfactory for original counts, providing that the checking were done by trained personnel. In the present studies, the original and check examinations were always made by different workers, and the checking was done by personnel temperamentally suited to this kind of work.

A satisfactory arrangement for complete checking was to have the original worker place the moss and debris on a cloth or tarpaulin by the quadrat, handful by handful as examined. The checker thoroughly examined the exposed bottom and walls of the quadrat first, and then the pile of moss and debris was worked over again, handful by handful, returning it to the hole. The average time required for original and check examinations of one-square-foot quadrats on plot VII, where population was dense and the moss deep, was 46 and 36 minutes respectively; on plot VIII, where population was lighter and moss more shallow, the averages were 23 and 16 minutes respectively.

The proportions of cocoons in the different categories overlooked in the first examination, results of all workers being pooled, are shown in Table XVI. There was no consistent relation between the percentages missed and density of population, or depth of moss, but within each category, the percentage was quite consistent between plots and years. This indicates that failure to recover the different kinds of cocoons equally well was due to certain intrinsic qualities of the cocoons, such as colour, shape, feel, and sound.²

The relative proficiency of different workers also varied, depending possibly upon eyesight, manual dexterity, and degree of interest in

²Emerged and chewed cocoons are often detected in the moss by the hollow sound produced when the cocoons happen to be pressed between the fingers.

TABLE XVI

PERCENTAGES OF COCOONS IN THE DIFFERENT CATEGORIES MISSED IN THE FIRST EXAMINATION

Data for 200 one-square-foot quadrates for each plot and year, and original counts made with fore-knowledge of checking.

Plot no.	Year	Total cocoons per square foot	Sound	Emerged	Killed by mammals	Killed by insects	Dead	Total
VII	1938	98.52	8 4	12 4	11 2	17 0	24.9	12.4
	1939	101.16	8 8	12 8	11.9	17 3	23 2	13 1
	1940	102 05	6 8	9 7	10 1	10.4	16 8	10 1
VIII	1938	7 34	7.0	10 1	13 7	7.4	23.1	9.4
	1939	13.12	6 4	17.7	17 3	13 3	21.9	12.3
	1940	14 55	8 1	13 9	17.9	13 8	9.5	14 0

the investigations. This variability is illustrated by the data in Table XVII. Experience was not consistently related to proficiency, as some workers did their most proficient collecting in their first year (e.g., *B*), while others varied but little from year to year (e.g., *A*, *F*, and *H*).

Some of the earlier results suggested a reduction in proficiency with increased fatigue, and for a thorough test of this possibility the 1940 data for plots VII and VIII were segregated into four parts, representing quadrates examined during the first and second half of the morning and afternoon respectively. The number of quadrates taken by each of four workers during each of the four periods was sufficiently great to test the individual reaction to fatigue, and the entire data

TABLE XVII

VARIATION IN THE PROFICIENCY OF DIFFERENT WORKERS IN RECOVERING SPRUCE SAWFLY COCOONS FROM THE FOREST FLOOR

Original counts made with fore-knowledge of checking.

Plot no.	Year	Percentage of total cocoons missed by the various workers									
		A	B	C	D	E	F	G	H	I	J
VII	1938	12 3	12.8	17.5	5.6	8.7	...	11.0
	1939	9.1	18.7	..	8 4	9 8	...	14 3	...	8 2
	1940	13.1	12.7	...	14.5	5 4	7 1	.	9.0	..	8.7
VIII	1938	5 1	27.3	14.0	4 8	8.9	8 1	11.9	8 8	
	1939	13.7	16.4	13 0	.	12 3	9.8	10 4
	1940	10.3	23 4	7.9	17 9	3 7		10.6	12.1	12 4

for each period served as a measure of the average reaction of all workers combined. The results (Table XVIII) do not support the view that proficiency drops with increasing fatigue.³ In fact, among the most striking points illustrated in the table, the remarkable consistency at all periods of the day shown by *H*, and the increased proficiency of *J* during the fourth period in plot VIII, refute the hypothesis in question.

The psychological effect of fore-knowledge of checking influenced the proficiency of all workers. This may be illustrated by comparing

TABLE XVIII

RELATIVE PROFICIENCY OF WORKERS AT DIFFERENT PERIODS OF THE DAY, BASED ON DATA FOR PLOTS VII AND VIII, 1940

The figures relate to the percentage of total cocoons (sum of the various categories) missed in the original counts.

Plot no.		Individual workers				All workers combined
		A	B	H	J	
VII	Morning, 1st half	12 0	12 5	9 1	8 4	9 8
	2nd half	9 0	11 9	8 7	8 7	9 3
	Afternoon, 1st half	13 3	13 6	8 7	6 5	10 0
	2nd half	16 2	12 7	9 5	10 3	11 6
VIII	Morning, 1st half	10 9	22 8	11 9	23 2	16 3
	2nd half	9 1	29 6	12 0	23 0	14 5
	Afternoon, 1st half	12 2	25 3	9 5	11 0	14 2
	2nd half	7 5	10 5	8 1	2 0	8 5

data for plot VII in 1937 and in 1938 (Table XIX); the original counts in 1937 were completed without any indication that checking might be done, while in 1938 it was understood by all workers that complete checking would be done. Fore-warning of checking improved the proficiency of every worker, and affected also each category of cocoon, though the sound cocoons were least affected.

From the data presented it seems clear that application of one or more correction factors to unchecked data on cocoon population, in order to bring the figures to a desired degree of precision, would be quite impracticable and arbitrary. Because of the variability between

³The question of the existence of fatigue attending prolonged effort should present no doubts to those with extended experience in sampling work of this kind.

the different types of cocoons, between workers and in some instances within workers at different periods in a wholly unpredictable manner, an attempt to standardize the percentage error would be just about as onerous an operation as the check counts themselves. Application of correction factors beyond the immediate scope of their origin would raise serious questions regarding the validity of such a procedure.

SUMMARY AND CONCLUSIONS

(1) The investigations were designed to test various methods of estimating populations of the European spruce sawfly in Eastern Canada, in order that reliable data relating to the population trend, effect of parasites and predators, etc., might be obtained.

(2) The first sampling technique consisted in determining the

TABLE XIX

EFFECT OF THE FORE-KNOWLEDGE OF CHECKING UPON PROFICIENCY IN RECOVERING SPRUCE SAWFLY COCOONS FROM THE FOREST FLOOR

Original counts in 1937 were made without fore-knowledge of checking, those in 1938 with knowledge of checking. Data for plot VII, central Gaspé.

Year	Percentage of cocoons missed, all workers combined						Percentage of total cocoons missed by individual workers		
	Sound	Emerg'd	Killed by mammals	Killed by insects	Dead	Total	E	F	H
1937	8 9	26 7	20 8	23 2	31 3	22 0	19 6	16 0	24 7
1938	8 4	12 4	11 2	17 0	24 9	12 4	5 6	8 7	11.0

number of cocoons in 2' X 2' quadrates spaced at fixed intervals in plots established in representative forest types, without regard to the suitability or otherwise of the location of the individual quadrates. The method thus aimed at estimating the population of the "whole forest" in the different forest types. Variability was so great, even in nearly pure black spruce stands, and the number of sample units required for moderate precision so beyond the capabilities of a field crew, that the method was abandoned.

(3) The second sampling technique consisted in determining the number of cocoons in 2' X 2' quadrates located within a restricted universe, which was defined as the area of suitable ground cover lying under dominant and codominant spruce trees in the forest types selected for study, with the further restriction that the quadrate

should lie within the band between one foot from the trunk and the vertical projection of the crown margin. This method aimed at estimating the cocoon population of that part of the spruce forest most closely related to the source of the cocoons. Variability was considerably lower than in the first method, and although the precision was less than was desired, there was in most instances a significant correlation between sample units taken under the same trees at different periods. This had the effect of reducing the standard errors of the mean differences between samples from the same plot, by an average of about 23 per cent in three plots considered here.

(4) The third sampling technique differed from the second in that the four-square-foot unit representing each tree at each sampling period, was made up of four equal sub-samples distributed within the universe under the tree. This provided a better estimate of the population under the individual trees, reduced variability of the sample as a whole, increased the degree of correlation between sample units taken at different times, and provided for further reduction in the standard errors of the mean differences for the individual plots.

(5) The intra-tree variability in cocoon density was significantly less than inter-tree variability in seven samples from two plots.

(6) Analysis of the sample data obtained by the third method, assuming the 200 sub-samples to represent independent variates, with consequent loss of correlation between the units of successive samples from a given plot, showed a higher variability of the one-square-foot quadrates, a proportionately smaller standard error of the mean due to four-fold increase in N , but in the majority of cases a reduction in the precision of mean differences between successive samples, as contrasted with the method of pooling the sub-samples under the individual trees. It is concluded that where successive samples in the same plot are contemplated, and where correlation is moderately high, the method of pooling a number of sub-samples taken under each tree to give an estimate for the tree as the sampling unit, is superior to taking the same number of quadrates without regard to their disposition within the sampling universe.

(7) The distributions obtained by the third sampling method were investigated for two plots over a three-year period. In one plot, the distributions conformed neither with the Poisson series nor with the normal distribution, but were consistently positively skewed, and leptokurtic in two out of three instances. In the second plot, two of the three distributions showed no significant departure from normality; the third distribution was positively skewed.

(8) Data relating to the proportions of the different categories of cocoons which were missed in the first examinations, and to the variability between workers and in the performance of individual workers at different times, indicated the impracticability of using correction factors to account for cocoons missed in the sampling. For this reason, all sub-samples taken by the third method were completely checked by another worker, bringing the counts to a satisfactory degree of accuracy. This varied slightly for the different types of cocoons, but averaged from about 96 to 98 per cent.

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SOME ASPECTS OF THE EFFECTS OF INTRAVENOUS
INJECTIONS OF ACETYLCHOLINE ON THE CENTRAL
NERVOUS SYSTEMBy GEORGE W. STAVRAKY¹

Presented by FREDERICK R. MILLER, F.R.S.C.

INTRODUCTION

THE discovery of local hormones, or chemical transmitters of nerve impulses by Otto Loewi (24) in 1921, gave new impetus to the study of the mode of action of nerves. Following this, one of the first proofs of the possibility of humoral transmission of nervous excitation from one organ to another in an intact animal was presented to this Society by Babkin, Alley, and Stavraky (1). Sir Henry Dale (13) drew attention to the similarity between the action of acetylcholine and that of the parasympathetic nerves; a great deal of work carried out by Dale and his associates,² as well as other investigators, elucidated the important part which acetylcholine, or acetylcholine-like substances, play in the transmission of nerve impulses from parasympathetic nerve endings to various organs, from somatic nerves to striated muscles and across synaptic membranes of autonomic ganglia. More recently, interest has been taken in the action of acetylcholine on the central nervous system; Schweitzer and Wright (34), Bülbring and Burn (9), and others have investigated its effects on the central transmission processes in the spinal cord. The last two workers have demonstrated that acetylcholine has predominantly an excitatory action when not combined with anticholinesterases, and that its effects are abolished by atropine. Worzniak and Gesell (37), Hansen, Worzniak, and Gesell (18), and Gesell and Hansen (16) studied the action of acetylcholine on the respiratory centre and found that both on intra-arterial administration and on local application, acetylcholine produces hyperpnoea of a highly coordinated type. Dikshit (14), Henderson and Wilson (19), and Benetato (5), showed that acetylcholine excites hypothalamic autonomic

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²Reviews of the literature on the subject may be found in the following contributions: G. L. Brown, Transmission at nerve endings by acetylcholine. *Physiol. Rev.* 1937, 17: 485. L. Goodman and A. Gilman, *The Pharmacological Basis of Therapeutics* (New York, 1941), chaps. xix, xx, pp. 317, 348.

nuclei, while Bonnet and Bremer (7), Miller, Stavraky, and Woonton (26), McKail, Obrador, and Wilson (28), Chatfield and Dempsey (12), Moussatché and Dias (27), and Brenner and Merritt (8) demonstrated, by a variety of methods, that acetylcholine enhances the activity of the cerebral cortex.

In the meantime W. B. Cannon (10) reviewed numerous instances of sensitisation to chemical stimulating agents; discussing the phenomenon of sensitisation, Cannon formulated a "Law of Denervation." He applied this law to the nervous system as well as to the peripheral structures, and expressed it in the following manner: "When, in a series of efferent neurones a unit is destroyed, an increased irritability to chemical agents develops in the isolated structure or structures, the effect being maximal in the part directly innervated."

According to this "law of denervation," removal of parts of the brain should make the remaining portions, which were in direct communication with those removed, more susceptible to stimulation by acetylcholine than the rest of the central nervous system. With this in mind, it was decided to remove various portions of the brain in aseptic operations, wait for the complete recovery of the animals and then, by injecting them with small doses of acetylcholine, try to get a selective stimulation of parts of the central nervous system sensitised by the removals. Some of the results obtained by this method will be discussed.

METHODS

In 10 cats, various parts of the brain were removed in aseptic operations ranging from one frontal lobe to a complete supratentorial decerebration. One frontal lobe was excised in 4 animals, the motor cortex being included in the removal. Portions of gyrus suprasylvius anterior and gyrus ectosylvius anterior were taken out as well as the more rostral portions of the cerebral hemispheres. The resection line passed through the neighbourhood of the sulcus ectosylvius anterior. In all the animals, the removals extended to the base of the brain, well in front of the optic chiasma, corresponding approximately to sections 3 to 5 in the atlas of Winkler and Potter (36).

In 3 cats, bilateral removals of the frontal lobes were carried out. In 2 cats, complete semidecerebrations were performed. The incision in this operation was made in the sagittal plane through the corpus callosum and the diencephalic structures about 2 to 3 millimetres off the midline, so as not to damage the optic chiasma, the stalk of the hypophysis cerebri and the hypothalamic nuclei of the opposite side.

In the transverse plane, the incision was made immediately in front of the superior colliculus. One cat with a complete supratentorial decerebration was kept alive almost 2 weeks. Of 10 animals which were operated on for survival experiments, 8 were eventually sacrificed, and the extent of the removals of various parts of the brain was verified both macroscopically and by histological sections.

The injections of acetylcholine bromide (Eastman Kodak) dissolved in about 0.5 c.c. of distilled water were administered at first once a week, later at longer intervals. The quantities of acetylcholine referred to in the text are given in milligrammes per cat, since the sensitivity of the animals to acetylcholine was found not to be closely related to weight. The weight of the operated cats varied from 2.4 kgms. to 3.8 kgms. excepting for those semidecerebrated, which were both lighter (1.9 and 2.3 kgms.). The injections were carried out without anaesthesia or previous preparation of any kind. The inner surface of the thigh was kept shaved and during the experiment the animals were placed on the side, the femoral vein was compressed with a finger by an assistant, and the injection carried out into the femoral or the great saphenous vein without any discomfort to the animal. The animals showed no ill effects from this treatment and several were kept alive for over a year. In addition to this, 7 sacrifice experiments were carried out involving decerebrations at various levels.

RESULTS

Effect of Acetylcholine in Intact Cats. As controls, more than 30 normal full-grown cats were injected with acetylcholine bromide. 0.1 to 0.2 mgs. of acetylcholine do not cause much motor activity. After 14-23 seconds latency, which is the same for all injections, there is a brief phase of unsteadiness or some slight vermiform movements of the extremities; the cat then lies down and lowers its head. A slight transient dilatation of the pupils occurs in the initial stage of the response and some salivation is observed. Injections of 0.2 to 0.3 mgs. of acetylcholine, which are most commonly employed in this study, cause slow spastic contractions of the extremities, which resemble vermiform movements of an athetoid type. In sensitive animals, these movements may end in a short phase of rigidity described in the next paragraph. After a slight initial dilatation which precedes the motor manifestations the pupils remain constricted in a bright room and acquire a glassy stare.

Injections of 0.3 to 0.6 mgs. of acetylcholine usually produce sharp generalized convulsions. The animal falls down in a typical tonic state;

the head is slowly drawn backwards on the neck and the extremities thrust out in a spasm. This may be followed occasionally, by a short clonic phase. Dilatation of the pupils, salivation, lacrimation, audible gastrointestinal motility, and occasionally defaecation accompany the convulsion. A severe seizure of this type may be followed by a period of depression during which the animal lies on its side, limbs being flaccid, pupils constricted and the breathing stertorous in type. About 2-3 minutes after the injection the cat recovers completely.

Ether anaesthesia or intramuscular administration of 1.0 mg. per kg. bodyweight of atropine sulphate prevents the onset of motor manifestations caused by acetylcholine. During deep ether anaesthesia, acetylcholine causes only a slight increase in tone comparable to its effect on decerebrate animals. Atropinization completely precludes the action of acetylcholine in the quantities used during the present investigation. These effects of anaesthesia and atropinization hold true for frontal-lobectomized and senidecerebrated cats as well as for the intact animals.

Effect of Acetylcholine After Removal of One Frontal Lobe. After the removal of one frontal lobe (including the motor cortex) the cats show contralaterally a persistent decrease of blinking and of fine movements of the ear, there is also a slightly brisker dilatation of the pupil and some disturbance of the "hopping and placing reactions" (2).

In the early stages of recovery from the operation (10 days-2 weeks) the most prominent features of the reaction of the animals to the administration of acetylcholine are a markedly greater initial dilatation of the contralateral pupil, slight lacrimation on that side, and an increased tendency to convulsions. However, gradually a completely asymmetrical response to the injections of acetylcholine develops on the two sides of the body. This becomes marked in 5-6 weeks after the operation and persists from then onwards.

Small doses of acetylcholine (0.05-0.1 mgs.) which were practically ineffective before the operation produce, after a short period of latency, a forward thrust of the contralateral front paw, and turning of the head toward the side of operation. In addition to this, the cat often makes several complete turns, walking in circles toward the side of operation just like a senidecerebrated animal. These motor manifestations are preceded by a transient dilatation of the pupils, which is more pronounced on the side opposite to the removal of the frontal lobe.

Injections of 0.1 to 0.2 mgs. of acetylcholine cause a greater dilatation of the contralateral pupil, and a contortion of the whole side of the body opposite to the removal. The head is drawn down, and the face turned away by a powerful contraction of the sternocleidomastoid

muscle; the front paw is raised over the head and often the protruding claws sink into the skin behind the ear. The back paw is drawn forward in a semiflexed position, and the body of the animal is contracted, forming a curvature with the concavity away from the side of operation. There is a spasm of the muscles of the tail and often some erection of the hair over the spastically contracted extremities. This tonic contraction lasts from 4 to 5 minutes and towards the end a coarse tremor sometimes appears in the rigid limbs. Gradually the effect wears off;

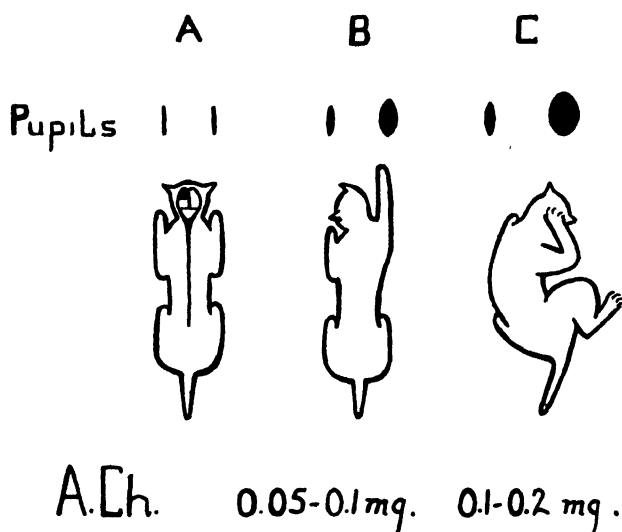


FIGURE 1.—The figure of a cat following the removal of the left frontal lobe, including the motor cortex, showing the movements and postures assumed after injections of subconvulsant amounts of acetylcholine.

A.—Pupils and posture before the injection.

B.—Quick turning of the head to the left, and a forward thrust of the right front paw, preceded by an unequal dilatation of the pupils.

C.—Contortion of the right side of the body preceded by an unequal dilatation of the pupils.

but increased rigidity, repeated quick turns of the head toward the operated side, and asymmetry of the pupils (the contralateral one being slightly larger) may persist for several hours.

The injection of 0.2-0.3 mgs. of acetylcholine sometimes causes normal slow vermiform movements of the extremities to develop on the operated side simultaneously with a tonic stiffening of the opposite side of the body. On the other hand, often the whole animal goes into a

short tonic convulsion which ends in a state resembling a transient decerebrate rigidity; the duration of this convulsion is measured in seconds, and it is over by the end of the first minute after the injection (Figs. 2 and 3, Plate I). The initial dilatation of the contralateral pupil is quite marked and there is a moment preceding the tonic convulsions when the ipsilateral pupil is small while the contralateral one is dilated.

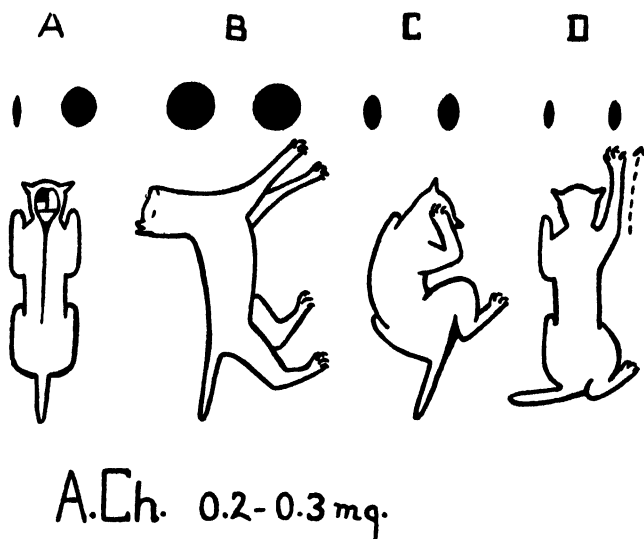


FIGURE 2.—Sequence of effects which takes place after an injection of a minimal convulsant amount of acetylcholine following the removal of the left frontal lobe including the motor cortex.

- A.—14 to 16 seconds after the injection. Initial unequal dilatation of the pupils which precedes the tonic convulsion.
- B.—45 to 60 seconds after the injection. Rigid state with a marked dilatation of the pupils.
- C.—1 minute and 30 seconds to 6 minutes after the injection. Contortion of the right side of the body with a slight inequality of the receding pupils.
- D.—6 to 10 minutes after the injection. Grasp reflex, some rigidity on the right side of the body, and a very slight inequality of the pupils.

During the convulsion both pupils become enlarged. When the animal comes out of the rigid state, the ipsilateral limbs acquire quickly a normal posture, and regain their natural tone, whereas the opposite side of the body goes into a characteristic contortion (Figs. 4, 5, and 6, Plate I). This unilateral contraction of the body which was described in detail in the preceding paragraph (effects of 0.1-0.2 mgs. of acetylcholine)

lasts up to 6-7 minutes. During the period of recovery, the animal often makes two or three violent grasping movements with the front paw, sinking its claws into the wood of the table in front of it (Fig. 7, Plate I). These grasping movements are automatic, they can be brought about in the paw by pressure on its pad and resemble closely the "grasp reflex" described in monkeys and humans with corresponding lesions of the brain (31, 35, 15, 6, etc.).

When the amount of acetylcholine injected is excessive, the initial tonic state may be followed by a generalized convulsion which, when severe, leaves the animal in a state of prostration. This can reduce and mask the unilateral contraction of the denervated side of the body.

Effect of Acetylcholine After the Removal of Both Frontal Lobes. After the removal of both frontal lobes, cats are restless, dirty, and must be fed for a considerable period of time after the operation. This is in agreement with the description given by Langworthy and Kolb (23), and Magoun and Ranson (25).

In cats which have both frontal lobes removed in one stage, the response to acetylcholine is marked and symmetrical on the two sides of the body. The predominant features of the response are an exaggeration and prolongation of the tonic convulsion, and a great accentuation of all the features of sympathetic excitation. After the usual latent period, an injection of 0.2-0.3 mgs. of acetylcholine causes a brisk dilatation of both pupils; this is quickly followed by a marked tonic convulsion during which the cat falls on its side with back arched, head retracted, pupils maximally dilated, all four extremities rigidly extended, tail raised high in the air, and the hair standing erect all over the body, in a characteristic fighting posture. At the end of this tonic spasm, the head is gradually lowered and the front paws are raised over the head; this phase of the response is usually more powerful, but less protracted than in the case of unilateral removal of the frontal lobes, although pronounced rigidity persists for from 4 to 6 minutes.

In one animal, the left frontal lobe was removed first and 13 weeks later, when injections of acetylcholine produced typical contractions of the opposite side of the body, the right frontal lobe was also taken out. As seen on Figs. 1-4, Plate II, three weeks after the removal of the second frontal lobe there was still considerable asymmetry in the response of the animal to injections of acetylcholine. In the later stages of the convulsion the cat lowered its head towards the right side of the body, and the right front paw moved much higher towards the back of the head than did the left one. At the end of the fifth week the response to acetylcholine became symmetrical though slightly quicker on the

side first operated on, the tonic convulsion being terminated by the placing of both front paws behind the head.

This form of experiment demonstrates advantageously, the gradual development of sensitisation to injections of acetylcholine which takes place after the removal of the frontal lobes.

Effect of Acetylcholine After Semidecerebration. In semi-decerebrated animals, the contralateral pupil remains permanently slightly larger than the ipsilateral one, the cats walk in circles towards the operated side and, as noted by Bard (3), show a greater disturbance in the "hopping and placing" reactions than animals with a unilateral removal of the frontal lobe. These animals are very sensitive to acetylcholine. As shown in Figs. 5, 6, 7, and 8, of Plate II, after the injection of 0.1 mgs. of acetylcholine, the animal falls over on its side with all four limbs rigidly extended, claws protruding, and pupils widely dilated. It is interesting to note that in these animals too, before the onset of the motor manifestations, the contralateral pupil dilates more widely than the one on the operated side. Towards the end of the convulsion a slow tonic contraction of the muscles develops in the extremities on the opposite side of the body, and is accompanied by slight rhythmic movements; the front paw is raised, and is gently worked up and down; the hind limb is held rigid in a semiflexed position, and a movement in it develops which suggests an incomplete scratch reflex (Fig. 4, Plate III). Contractions reach their maximum strength within 2-4 minutes after the injection; the front paw is drawn up rigidly under the chin; but not behind the head as in frontal-lobectomized animals; also there is no spinal curvature and no head depression.

Injections of 0.2 mgs. of acetylcholine have a marked effect, the resulting convulsion being of a severe and protracted nature. Sometimes the cat suddenly jumps into the air and then falls on to the table in a vigorous bout of activity; tonic and clonic convulsions follow, the teeth chatter, the bowels move, and every function and muscle in the body appears to be in action. When the seizure is over, the animal is left in a state of prostration, and lies for some time panting and exhausted.

Effect of Acetylcholine in Decerebrated Cats. In a cat with a complete supratentorial decerebration, several injections of acetylcholine caused an increase in decerebrate rigidity. In order to confirm this observation, in seven sacrifice experiments, cats were decerebrated, and after the animals recovered from the operation, intravenous injections of acetylcholine were given. Injections of 0.5-1.0 mgs. of acetylcholine produced an increase in the decerebrate rigidity. This increase in rigidity was transient and was followed by a period of hypotonicity which lasted

from 3 to 5 minutes. Smaller doses of acetylcholine (0.1-0.3 mgs.) produced only a transient decrease in tone.

DISCUSSION

The unilateral responses to intravenous injections of small doses of acetylcholine, seen during the experiments on the frontal-lobectomized and semidecerebrated animals favour the view that a sensitisation to stimulating agents is present in parts of the nervous system which were connected with the excised regions of the brain. The assumption that the sensitisation occurs pre-eminently within the central nervous system is based on the fact that anaesthesia abolishes most of the responses caused by injections of acetylcholine. Cannon and Haimovici (11) found that the sensitisation of spinal neurones to acetylcholine following semisection of the spinal cord takes place within 5-14 days after the operation. The full effect of injections of acetylcholine following removal of a frontal lobe, appears only 5-6 weeks after the operation.

In dealing with the effects of acetylcholine it is recalled that in large quantities it is a convulsant agent (21, 33, 8). The operated cats show an increased tendency to convulsions after the injections of acetylcholine, and these convulsions are of a more severe nature than in intact animals. Lowering of the threshold of the motor cortex to electrical stimulation after the removal of other large areas of the cerebral hemispheres has been described by Bard (2). Cannon (10) regards this as indirect evidence of the sensitisation of the motor cortex. Our results may be interpreted from the same point of view and show that a sensitisation of the remainder of the brain to chemical agents results after the removal of large parts of the brain. It is interesting to note that the convulsions in the operated cats involve both sides of the body almost symmetrically, but whereas the seizure which takes place on the same side as the removal is over usually within 45-60 seconds after the onset, that on the side opposite to the removal passes into a characteristic contortion which often lasts for several minutes. This type of response is particularly marked in the frontal-lobectomized animals. The contractions which are localized to the side of the body opposite to the removal can be reproduced without any ipsilateral response by injecting small quantities of acetylcholine; still smaller amounts of acetylcholine produce quick co-ordinated movements which are again localized to the opposite side in the frontal-lobectomized cats. This shows the great degree of sensitisation which takes place in certain parts of the central nervous

system after the removal of one frontal lobe and points to the variety of regions associated with it.

The different postures and movements of the frontal-lobectomized and semidecerebrated animals already described, following the injections of acetylcholine, further support the view that the sensitisation involves different levels of the central nervous system.

The sympathetic responses caused by the injections of acetylcholine in the frontal-lobectomized animals are particularly marked after bilateral removals of the frontal lobes. This is in keeping with the view that the sympathetic nervous system has representation in the rostral parts of the cerebral hemispheres (17, 30, 22, etc.).

Of interest is the appearance of a grasp reflex in the cats with unilateral removals of the frontal lobes during the subsiding stages of the response to injections of acetylcholine. The appearance of a grasp reflex in intact monkeys during the action of various pharmacological agents has been studied by Richter and Patterson (32); this was attributed to a depressing effect of the drug on the frontal lobes. In our experiments, the reflex is probably due to a stimulation by acetylcholine of the sensitised parts of the central nervous system.

The effect of injections of acetylcholine on decerebrate rigidity deserves to be mentioned. The fact that either depression or excitation of the extensor tone can be brought about by the injections of different quantities of acetylcholine, is comparable to the results of other investigators who studied the action of acetylcholine on the central nervous system.

The mechanism of the action of acetylcholine, when injected intravenously into non-anaesthetized animals is difficult to analyse, and only indirect inferences regarding it can be drawn. Local application of acetylcholine to the cortex of the cerebral hemispheres may result in motor activity. This was shown by Miller, Stavraky, and Woonton (26), in the eserinated cat under light dial anaesthesia, while Moussatché and Dias (27) have succeeded in producing regular epileptiform convulsions by an application of acetylcholine to the motor cortex of dogs under the influence of morphine. Cannon and Haimovici (11) have analysed the underlying mechanism responsible for the contraction of the quadriceps muscle of the semisected side of the body and found that a true sensitisation of the nerve cells to acetylcholine takes place below the semisection, also there is present some sensitisation of the muscle after the destruction of the penultimate neurones. These facts favour the possibility of a direct action of acetylcholine on the central nervous system. On the other hand, marked circulatory changes caused by injections of acetyl-

choline, might lead to a transient asphyxiation of the brain, thus causing excitation of the central nervous system and possibly even inducing the convulsion, in the case of large doses of acetylcholine. This latter possibility seems improbable on account of the brevity of the latent period elapsing between the injection and the onset of the motor manifestations. However, a frontal-lobectomized cat, placed in a low-pressure chamber, and suddenly deprived of oxygen at the altitude of 40,000 feet above sea level, develops a mild convulsion during which some asymmetry is evident. Also, it is known that injections of acetylcholine can cause liberation of adrenaline (4); besides complicating the effect of acetylcholine on the cardiovascular system, adrenaline could influence the action of acetylcholine on the central nervous system in a more direct way. Combined with absinth, adrenaline increases the severity of convulsions caused by this latter drug (29, 20, 21), whereas administered in very large doses it may induce convulsions even by itself.

The fact that atropinization prevents the onset of motor manifestations caused by intravenous injections of acetylcholine does not help to clarify this point, and on the whole it is felt that the evidence is in favour of a direct stimulation of the central nervous system by acetylcholine, but that other factors may be involved in the reaction.

SUMMARY AND CONCLUSION

(1) A study is presented of the effects of intravenous injections of acetylcholine in unanaesthetized cats in which parts of the brain have been removed in aseptic operations.

(2) The removal of one frontal lobe, including the motor cortex, leads to an asymmetrical response to injections of small quantities of acetylcholine (0.05-0.2 mgs.) which becomes fully established 5-6 weeks after the operation and consists of: characteristic quick movements, tonic contractions, a typical posture, and various manifestations of unilateral excitation of the sympathetic nervous system. These reactions all take place on the side of the body opposite to the operation. Large doses of acetylcholine (0.2-0.3 mgs.) produce generalized convulsions which increase in severity with the removal of large areas of the brain.

(3) After a bilateral removal of the frontal lobes, acetylcholine causes a typical symmetrical response on both sides of the body, which is characterized by a great exaggeration of all the signs of sympathetic excitation.

(4) A particular unilateral response is also produced by the injection of acetylcholine in semidecerebrated cats which can be dis-

tinguished from the one elicited by acetylcholine in frontal-lobectomized animals. This response is best studied after the termination of a brief generalized convulsion to which these animals are very susceptible.

(5) Most of the effects of acetylcholine described are precluded by anaesthesia or atropinization of the animals.

(6) The results are interpreted as being due to a sensitisation to chemical stimulation of residual parts of the central nervous system after the removal of upper links in the chains of descending neurones.

My thanks are due to Mrs. C. C. Calder for the diagrams and to Mr. D. B. Ferguson for the photography.

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EXPLANATION OF PLATES

PLATE I

The effect of an intravenous injection of 0.25 mg. of acetylcholine in a cat in which the left frontal lobe, including the motor cortex, was removed 4 months previously.

FIGURE 1.—Control before the injection.

FIGURE 2.—20 seconds after the injection. Generalized tonic convulsion.

FIGURE 3.—45 seconds after the injection. Phase which resembles transient decerebrate rigidity.

FIGURE 4.—1 minute and 30 seconds after the injection. Beginning of the unilateral contraction.

FIGURE 5.—2 minutes and 30 seconds after the injection. Raising of front paw over head.

FIGURE 6.—3 minutes and 45 seconds after the injection. Contortion reaches maximum intensity.

FIGURE 7.—5 minutes and 30 seconds after the injection. Time when grasp reflex is seen. (It is more marked in the early stages of recovery after the operation.)

FIGURE 8.—7 minutes and 30 seconds after the injection. Stage of decreasing rigidity.

PLATE II

FIGURES 1, 2, 3, and 4.—The effect of an intravenous injection of 0.3 mg. of acetylcholine in a cat in which the left frontal lobe was taken out 4 months previously. The right frontal lobe was removed 3 weeks before the injection.

FIGURE 1.—Control before the injection.

FIGURE 2.—25 seconds after the injection. Generalized tonic convulsion with arching of the back and erection of hair.

FIGURE 3.—45 seconds after the injection. Right front paw is raised over the head but not the left one.

FIGURE 4.—55 seconds after the injection. Maximal lowering of the head. (1 minute and 20 seconds after the injection the right front paw was removed from the head, but the bilateral rigidity lasted 6 minutes and 30 seconds.)

The injections were repeated 2 and 3 weeks later and resulted in a symmetrical response. (Both front paws were raised over the head of the animal.)

FIGURES 5, 6, 7, and 8.—The effect of an intravenous injection of 0.1 mg. of acetylcholine in a semidecerebrated cat 3½ months after the operation. (The left cerebral hemisphere was removed.)

FIGURE 5.—Control. Posture before the injection. Walks in circles and turns head of the animal.)

FIGURE 6.—20 seconds after the injection. Generalized tonic convulsion.

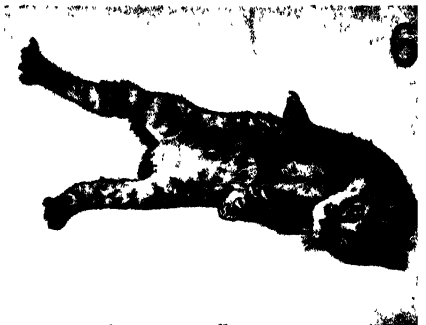
FIGURE 7.—45 seconds after the injection. End of the convulsion and beginning of raising of the right front paw.

FIGURE 8.—2 minutes after the injection. Note rigid front limb in a typical position: adduction at the shoulder, flexion at the elbow, and extension at the wrist. Hind limb is stiffened in a semiflexed position. (Effect lasted 4 minutes and 30 seconds after the injection.)

PLATE I



PLATE II



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SECTION V

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PRESIDENTIAL ADDRESS

LIFE CYCLES AND PHYLOGENY IN THE HIGHER FUNGI

By H. S. JACKSON, F.R.S.C.

A more descriptive and accurate title for the discussion which I propose to present this morning would have been the more cumbersome one: "Reflections on the Possible Bearing of Comparable Life Cycles of the Higher Fungi and Red Algae to the Problem of Fungus Phylogeny." The shorter title may lead my audience to anticipate that the problem of the phylogeny of the higher Fungi has been solved, or might suggest that your speaker has some very definite convictions on this subject. I assure you that this is far from the true situation. I propose merely to discuss certain possibilities with reference to the phylogeny of higher Fungi which have interested me and which centre in the evidence to be gained from an analysis of existing algal and fungus life cycles.

Any discussion involving phylogeny of the higher Fungi must of necessity be highly speculative. There is no help available up to the present time from the very meagre fossil record, nor is it likely that this source of information, which has proven so helpful in determining relationships in the animal kingdom and among vascular plants, will ever be of much help in unravelling the mysteries of fungus origin, because of the delicate and evanescent nature of these organisms. Whatever attempts at deduction are made with reference to fungus phylogeny must be based upon the present incomplete state of our knowledge of these organisms, and comparisons between groups suspected of showing relationship must be made on the basis of present-day forms.

It does not seem necessary to attempt at this time any review of the historical development of ideas with reference to phylogeny in the Fungi nor to analyse in detail the various views which have been put forward in support of special theories. These points have been well covered by others (Atkinson, 1915; Bessey, E. A., 1913, 1942; Dodge, 1914; Orton, C. R., 1927).

As a background for the present discussion it may be briefly stated that two opposing views are held by modern students of the Fungi. One

group would look for the origin of the higher Fungi (Ascomycetes and Basidiomycetes) among the Phycomycetes and would derive the Basidiomycetes from the Ascomycetes. Another group would derive the higher Fungi from the ancestral red Algae, usually accepting the Ascomycetes as the basic fungus group from which the Basidiomycetes are derived.

[I have, for a number of years, been intrigued by the problems arising from a consideration of the peculiar types of life cycle which form so characteristic a feature of the plant rusts (Uredinales). This interest finally culminated, a number of years ago, in the publication of a paper dealing with an analysis of the evolutionary tendencies within that group and the origin, one from another, of the various types of life cycle (Jackson, 1931). In that paper it was established, to my own satisfaction at least, that the long-cycled heteroecious and heterothallic rusts are the primitive ones, and that the micro- and endo-forms as well as the intermediate -opsis and brachy-forms, are all derived by reduction from the eu-forms.] This experience, together with the more recent one of teaching an introductory course dealing with the various groups of the Algae has served to direct my attention to the fundamental significance of life cycles in the evolution of lower plants generally.

[Recent advances in our knowledge of the rusts initiated by the experimental work of Craigie (1927 a & b, 1931) and developed by the cytological studies of Andrus (1931, 1933) Miss Allen (1930, 1932, a, b, etc.), Miss Rice (1933), Lamb (1935), and others, and by the observations of Craigie (1933) and Pierson (1933) and particularly those of Buller (1938, 1941) on the flexuous receptive hyphae emerging from the spermagonia (pycnia), have not only given us a clearer picture of the sexuality in the group but have served to direct our attention anew to the possible relationship of the rusts to the red Algae.] Then too, recent developments with reference to sexuality in the Ascomycetes centring in the experimental researches of Drayton (1932, 1934) on *Sclerotinia Gladioli*, Ames (1932, 1934) on *Pleurage anserina*, and Dodge (1932, 1935), Backus (1939), and others on the species of *Neurospora*, together with an ever-increasing number of recorded observations on the wide-spread occurrence of a spermatial (microconidial) type of sexuality in the Ascomycetes, have not only served to strengthen former concepts of a relation between Ascomycetes and Uredinales but for some at least have revived interest in the possibility of the origin of Ascomycetes from red Algae. On the other hand, recent investigations by Rosenvinge (1929, 1931, a, b), Bergesen (1927), Gregory (1930, 1934), and others, admirably summarized and interpreted by Svedelius

(1931, 1937) and Kylin (1935) have served to focus attention on a number of species of red Algae, in part formerly misunderstood and interpreted as parasitic, which now seem clearly to represent a series of short-cycled forms derived by reduction from those of full life cycle or in one case perhaps representing an intermediate step in a progressive evolution from the haplobiontic to the diplobiontic Florideae. This series of reduced or simplified algal species appears to exhibit a striking parallelism with certain microcyclic rusts in the manner in which the reduction has occurred and, furthermore, includes a type of life cycle in the red Algae which is essentially like the characteristic life-cycle type of the majority of the higher Ascomycetes.

It is these relatively recent developments in our knowledge of these three groups, Uredinales, Ascomycetes, and the Florideae, together with a deep-seated conviction that an analysis of life cycles should prove useful and perhaps highly significant in connection with the elucidation of the problems of phylogeny, which has induced me to venture upon a trial excursion into the realm of speculation with reference to their possible interrelations. For the moment, then, I have chosen, as others have done before, to view the rusts and the Ascomycetes from a background of a possible red algal ancestry. I should hasten to add that, in doing so, I do not wish to leave the impression that I have formed any irreversible convictions. [Our knowledge of the Fungi is so incomplete at present that it seems wise to keep a reasonably open mind with reference to their phylogeny.]

The reasons given by Linder (1940) for dismissing all consideration of a possible red algal background for the Basidiomycetes are not in my opinion sufficiently convincing. The objection that the red Algae are marine seems particularly inappropriate. With the whole picture of plant and animal evolution as a background, attempts to trace many of the groups to their ultimate place of origin is quite certain to lead to the sea. The facts that the chromosomes are many in the present-day red Algae and relatively few in the Basidiomycetes, and that the type of nuclear division in the two groups differs widely would appear to be more fundamental objections. While it is necessary to make comparisons between existing types, it is obvious that if the Basidiomycetes or Ascomycetes or both are to be derived from the red algal ancestors the origin must have occurred at a very remote period, perhaps having its beginnings before a land flora emerged. Who can visualize what the chromosome number or the particular type of nuclear division was at that period in the evolution of the groups concerned or what changes may have occurred during the long period of time since they began to

diverge?¹ The objection that the resemblance between the life cycles of the groups concerned is due to independent parallel development will be mentioned later.

To attempt to discuss the phylogeny of the Basidiomycetes and Ascomycetes in all its aspects or in any great detail does not seem appropriate for the present occasion. I shall therefore limit any detailed discussion to that phase of the subject which can be centred around a consideration of life cycles. To my knowledge, this phase of the comparison between Fungi and red Algae has never been adequately presented and the implications furnished by a comparative study of life cycles seem not to have been fully appreciated by those who choose to disregard the possibility of deriving the higher Fungi from the ancestral red Algae.

SEXUALITY IN THE FUNGI

The sexuality of the Fungi which I shall discuss has in the past been frequently misinterpreted. Though the correct interpretation has often been properly stated, there is evidence from recent literature that misunderstanding is still prevalent. It therefore seems desirable that a general statement of the situation, as I view it, be given at this point, before the comparisons of life cycles which I shall make are attempted.

In the discussions which follow, it will be necessary to use the terms heterothallic and homothallic quite frequently. The meaning of these terms has become considerably modified in recent years from the original definition provided by Blakeslee (1904) at the time he introduced these terms into botanical terminology. While Blakeslee found a need for the terms because he was working, at the time with *Mucor* species having an essentially isogametangial type of sexuality, his definition of heterothallism carried with it a definite implication with reference to the separation of sexes on different thalli. It was his intention to provide, for use in discussing sexual phenomena in gametophytes, a set of terms to replace dioecious and monoecious, which terms had originated in connection with sexual phenomenon as applied to diploid sporophytes. This is made clear in a later paper by Blakeslee (1906). The flowering plants, for example, might be either monoecious or dioecious as to the sporophyte but are always heterothallic as to the gametophytes. This situation has been further complicated by some authors who have con-

¹The intra-nucleolar type of mitosis described by Svedelius (1937) in *Lomentaria rosea* is suggestive that radically different methods of nuclear division may occur in the present-day red Algae.

tinued to use the terms monoecious and dioecious in discussing sexual situations in haploid thalli of thallophytes.

[In the Fungi and particularly in the higher Fungi, the term "heterothallic" has gradually come to be applied to the situation where two gametophytic (haploid) thalli, interacting sexually, are necessary to bring about the development of the perfect fructification. While a sex difference in these thalli has often been implied, too little attention has been given to considering whether the difference was actually due to segregation of sexes or to a difference due to segregation of factors controlling the union or association of sex nuclei, i.e., interfertility. As a result papers are still being published in which the authors use the expression "thalli of opposite sex" when it is abundantly evident that the species they are dealing with is properly to be interpreted as bisexual (hermaphroditic), self-sterile, and interfertile. This casual assumption of sex difference in organisms in which no sex structures are available as a guide to interpretation has resulted in the use of the expression "multiple sexes" to account for the occurrence of four, instead of the more usual two, interfertility groups in the mushrooms and related fungi. It may be interpolated here that there is deductive evidence at least, that the mushrooms are to be derived from ancestral forms which had a more orthodox sexuality and in which the heterothallism was of the interfertility type.

Similarly the term homothallic, originally introduced as a companion term to heterothallic by Blakeslee and to provide a term to replace hermaphroditic as applied to gametophytes is now used broadly in the higher Fungi to refer to any sort of situation where full fructification may be obtained as a development from a single spore—only one thallus being involved. The term has been in common use in the sense in which it was originally introduced by Blakeslee, in connection with fungi which are visibly sexually hermaphroditic and self-fertile as in many Phycomycetes and Ascomycetes having antheridia as the male structure. The term has also, unfortunately, been applied in connection with certain species of Ascomycetes (*Neurospora*, *Pleuroge*, *Gelasinospora*) which have four spores in the ascus, instead of the more usual eight, and which fruit readily from a single spore which contains two genetically different nuclei. That the nuclei are genetically different is shown when uni-nucleate spores, which occasionally occur as an abnormality, are isolated and paired together. Single nucleated isolates do not fruit. Fifty per cent of the paired ones do. These forms are then basically heterothallic—hermaphroditic, self-sterile, and interfertile. A perfectly comparable situation has recently been encountered in the Basidiomycetes.

Aleurodiscus canadensis normally produces two spores to the basidium and two of the four nuclear products of meiosis pass into each spore. Such spores, when germinated, produce clamps on the hyphae—evidence of ability to fruit. Three-spored basidia occasionally occur and then two of the spores receive only one nucleus. When such uninucleate spores are isolated and paired, proof is obtained that the species is basically heterothallic (Skolko, 1944).

In the majority of cases of homothallism in the higher Fungi where fructification is obtained from a single uninucleate spore, there is evidence of sexual degeneracy as, for example, in many short-cycled rusts where spermatia no longer are present, but in which the whole series of nuclear events characteristic of long-cycled heterothallic forms still occurs—nuclear association, fusion in the teliospore and reduction in the basidium. Since the nuclei which associate and ultimately fuse are sister nuclei, the reduction division has become merely a matter of form. The whole series of events would appear to represent a phylogenetic "habit" derived from heterothallic long-cycled forms (from which the short-cycled forms are to be derived by reduction) in which the process had some genetical significance (Jackson, 1935). Similar cases occur in the mushrooms (*Coprinus sterquilinus*).

It is necessary then to be sure of the type of heterothallism or homothallism with which one is dealing, and to define the terms in each case. In the discussions with which I shall deal, most of the cases of heterothallism which will be encountered in the Fungi are of the type involving interfertility phenomena and most of the cases of homothallism used are sexually degenerate and derived from heterothallism of the interfertile type.

Before I leave this discussion of sexuality I will venture the following generalization. Heterothallism, in whatever sense the word is used, is essentially a provision for hybridity. The loss of it often results in an extreme form of self-fertility (homozygosity). It is probable that homothallism of any type did not precede heterothallism in the evolution of sex but followed it. The condition which preceded heterothallism, in all probability, was not homothallism but sexlessness. Since homothallism is often indicative of sexual degeneration, the genetical implications of such a situation must not be ignored and since this condition is probably derived from heterothallism caution should be exercised in using such forms as a basis for phylogenetic discussion. They are useful in that connection only in so far as they may represent relic forms or as they may assist in an understanding of processes of simplification.]

COMPARISON OF A DIPLOBIONTIC RED ALGA WITH A
MACROCYCLIC RUST

In order to make clear the point of view which I wish to present, it will be necessary to review the basic types of life cycle in the various groups to be discussed. This seems simplest to do by means of diagrams and the first comparison (Fig. 1) will be between a long-cycled (diplobiontic) red Alga and a long-cycled rust. The red algal diagrams are borrowed bodily from Svedelius (1931) and the rust and Ascomycete diagrams are prepared in much the same form to allow for ready comparison.

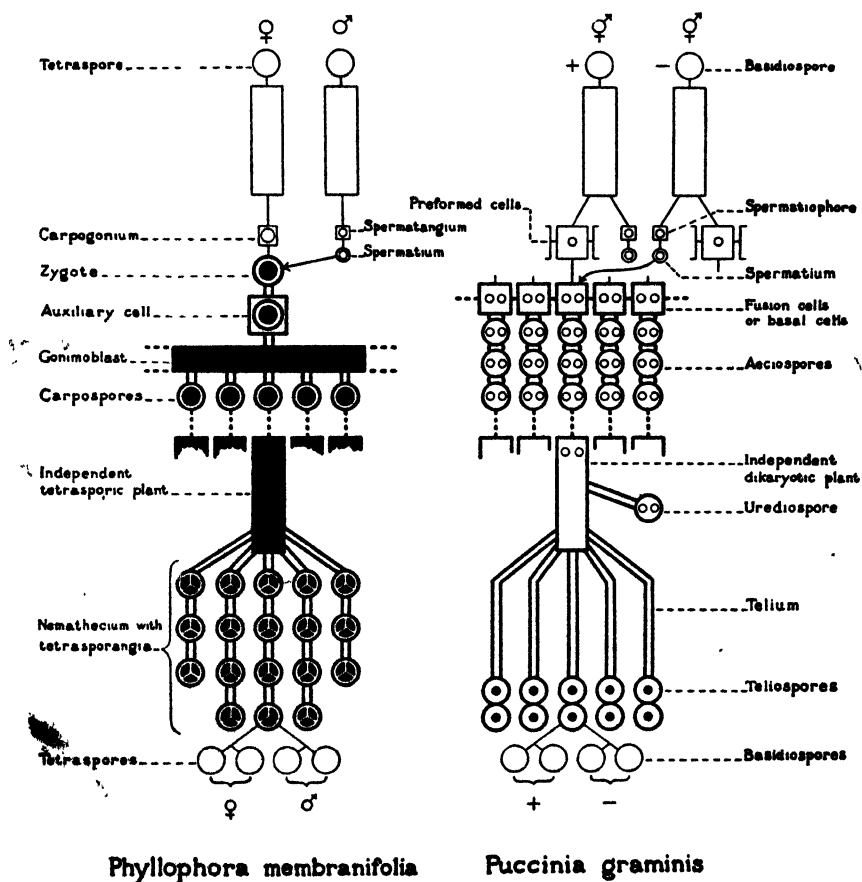


FIGURE 1.—Comparison between a diplobiontic red alga, *Phyllophora membranifolia*, with a typical heterothallic macrocyclic rust, *Puccinia graminis*.

For the red Algae Svedelius' diagram of *Phyllophora membranifolia* is used. Two sexually differentiated (sexually heterothallic) gametophytes are involved, one bearing the characteristic carpogonia with receptive trichogynes, the other bearing the non-motile spermatia, which are passively carried to the trichogyne. Fertilization is accomplished by the passage of the nucleus of the spermatium down the trichogyne and its union with the carpogonial nucleus. The zygote develops without resting to the diploid gonimoblast which bears diploid carpospores which are disseminated and develop into independent but somatically similar diploid plants. Reduction division (meiosis) occurs in the tetrasporangia borne on the independent portion of the diploid phase and four haploid tetraspores are formed enclosed in the tetrasporangium, which are ultimately liberated and develop into the sex plants. This is the essential situation in all the Florideae except the Nemalionales. *Phyllophora membranifolia* was selected rather than the better known genus *Poly-siphonia* because in *Phyllophora* the tetraspores are aggregated into a characteristic tetrasporic nemathecium, a development of the independent diploid phase, and also for reasons which will become evident when other comparisons are made.

So far as the life cycle is concerned, the most significant feature for the purpose of our discussion is that the diploid phase is in two parts, the gonimoblast with its carpospores and the independent tetrasporic plant. The portion represented by the gonimoblast is developed from the zygote in association with and nutritionally dependent on the mother haplont. This characteristic division of the diploid phase is unique and is known nowhere else among holophytic thallophytes. It should also be emphasized that the sex nuclei fuse in the carpogonium and all cells of the gonimoblast and the independent tetrasporophyte contain diploid nuclei ($2N$).

For the long-cycled rust to be used in comparison, I have selected *Puccinia graminis*, largely because it is well known and has been used extensively in studies on sexuality. The essential features of the diagram would serve for any long-cycled heterothallic rust.² In the diagram two bisexual haplonts are indicated as essential in sexuality. These develop parasitically in the barberry leaf and originate from infection by basidio-

²There are so many gaps in our knowledge of the mechanism of sexuality in the rusts and so much that is conflicting in the mass of literature dealing with this subject, that it is necessary, in order to present a connected picture in a few words, to make selections and even to resort to speculation. I have presented the general picture as I view it. With reference to the function of the emergent hyphae which have played so prominent a part in recent cytological investigations, I would not wish to imply that they may not occasionally function to receive spermatia. This

spores which have formed in the spring from germinating teliospores on overwintered wheat stubble or other grasses. Each haplont bears spermatia (pynciospores) in characteristic spermatogonia (pyncia) which form usually on the upper side of the leaf in association with the fundaments of the aecial (cluster cup) structures which develop on the lower side. No further development of the aecial fundament takes place unless compatible spermatia from another haplont are brought into position to be received by flexuous hyphae which develop from the walls of the spermatogonia, and are present in the nectar which is secreted in association with the mature spermatogonia, and to which insects have brought the compatible spermatia from another haplont. What follows has not been fully demonstrated, but there is reasonable evidence for the assumption that following contact with the flexuous hyphae and the spermatia, the nucleus of the latter passes down through the flexuous hypha, and finally finds its way by migration or by division and migration to one or more of a group or layer of preformed cells which is present in the aecial fundament and in which the *effective* association of the sex nuclei ultimately takes place. These "preformed" cells are the centre of activity in the aecial fundament and through fusion with other cells—the fusion cells of the cytologists—or through nuclear migration ultimately become the basal cells of the aeciospore chains. One of these nuclei has come in through the transported spermatium; the other is a somatic nucleus already present in the "preformed" cell. There is reason to suggest that this is a multiple process. A group of pyncia are usually formed from one infection and a number of aecial fundaments are formed, in a group on the under side of the leaf opposite to the pyncia. It may be presumed that many flexuous hyphae receive many compatible spermatia and the nuclei of these find their way to many of these "preformed" cells. This suggestion does not rule out the possibility of division of these nuclei during migration.

In any case, soon after there has been a transfer of spermatia from one monosporidial infection to another the aecial fundaments resume development and chains of aeciospores are formed directly or indirectly from the "preformed" cells referred to above, which are now binucleate.

I have reviewed the sexual process in the rust, as I visualize it, in

seems quite possible but the flexuous hyphae found so consistently by Buller, emerging from the spermatogonia into the nectar where compatible spermatia have been carried by insects, seems on general biological grounds to be acceptable as the primary mechanism for spermatization. Miss Ashworth (1935a) has shown that emergent hyphae occur in association with uredinia and telia of long-cycled rusts and in such short-cycled forms as *Puccinia malvacearum*. Under these conditions the function of such hyphae cannot be to receive spermatia.

some detail as it is obviously, in part, a substitute process and can be compared directly with that of the red Algae only after further speculation. The spermatia are obviously functioning as male cells are expected to function, the "preformed" cells referred to are in the position where the female structures should be, and they occur in numbers. If we may visualize that individually each represents what remains of a carpogonium-like structure, we may see in the aecial fundament a modified compound cystocarp which may be compared with the compound cystocarp of present-day Corallinaceae. It is significant that cytological evidence indicates that it is necessary that the two sex nuclei be present in these cells before any further development of the aecial structure takes place, and that genetical evidence supports the view that, barring abnormalities, the pair of nuclei in the aeciospore as well as those in the cells of the resulting independent dikaryophyte are actually derived from the two sex nuclei.

There is no fusion of the sex nuclei in these "preformed," now "fusion" or "basal" cells. They represent the position of the first *effective* association of the sex nuclei and the real beginning of the dikaryotic diploid phase of the rust cycle. The mature aecial structure with its chains of aeciospores may be compared with the gonimoblast of the diplobiontic red Algae. The aeciospores correspond to the carpospores and it may be noted that many red Algae develop carpospores in chains. This phase of the rust diplont is, like the red algal gonimoblast, developed in association with and primarily dependent upon the mother haplont. The aeciospores like the carpospores are disseminated and, in the rust, develop into an independent, parasitic, dikaryotic diploid thallus in the tissues of the wheat plant. Ultimately teliospores are formed, in the cells of which the nuclei finally fuse and reduction follows in the developing septate basidium. The haploid basidiospores are of two sorts which may designated + and —, the difference having been brought about by the segregation of factors governing interfertility. The two haploid thalli developed from the + and — basidiospores are not of "different sex" but are properly to be interpreted as bisexual (sexually homothallic)—"hermaphroditic, intersterile, and cross^{ed} fertile."

The diagrams bring out graphically the close correspondence of the life cycles in the two groups. The most significant feature of this correspondence is that the diploid phase is in two parts, as in the diplobiontic red Algae, one developed from and dependent upon the mother haplont and the other, independent. As pointed out previously, this type of cycle in the red Algae is unique among holophytic plants and is known nowhere in the Fungi except in the rusts.

The most significant difference in the cycles is in the nuclear character of the diplonts. In the rust, the sex nuclei do not fuse at the time they become associated but repeatedly divide in such a manner that, in the normal course, each cell of the rust diplont contains a product of the original associated pair which finally fuse just before reduction is to occur. That it is the products of the sex nuclei which finally fuse in the teliospores seems amply proven by genetical studies in this and other groups of higher Fungi where a dikaryotic diplont occurs. The simple explanation that the dikaryon has arisen through a delay in the fusion of sex nuclei seems the only acceptable one, and hence there should be no basis for objecting (Linder, 1940) to comparing this dikaryotic diplont ($N + N$) of the rust with that of the red Algae ($2N$) so far as the comparison of the cycle is concerned. Indeed, it may be stated parenthetically, that it has yet to be shown that there is any genetical difference traceable to the circumstance that in a dikaryotic diplont the two sets of sex chromosomes are dividing in mitosis in separate nuclei ($N + N$) while in the more orthodox diplont ($2N$) they retain their identity through mitotic divisions in one nucleus.³

The diagrams and the comparisons which have already been made will serve to focus attention on possible homologies between the two

³From the viewpoint which I choose to take, it is evident that the dikaryotic diplont of the rust and other Basidiomycetes may be compared with the F_1 generation of other organisms having a true diplont. Such genetical study as has been made of the dikaryotic F_1 in the Basidiomycetes supports the view "that the binucleate condition . . . does not in any way interfere with the expression of dominance." I am in full agreement with the statement made by Johnson and Newton (1940, p. 610) from which the above phrase is quoted. Among others, the papers by Miss Nobles (1935) on *Peniophora Allescheri* and Miss Macrae (1942) on *Panus stipiticus* are of interest in this connection. Dodge's statement (1939) that "The larger part of the fruiting bodies of mushrooms and Ascomycetes are haploid; therefore we should not use the term hybrid to describe a mere intermingling of hyphae or nuclei of two different races to form the framework of such structures," indicates a fundamental misconception of the difference between the fruiting bodies of these two groups. The mature fruiting structure of the higher Ascomycete is, to be sure, largely haploid, but the mushroom fruiting body is totally a product of the dikaryotic diploid phase, and with its supporting thallus is comparable to the dikaryotic diplont of the long-cycled rust. The corresponding phase, the true F_1 of the higher Ascomycetes, consisting as it does of the ascogenous hyphae—to the fusion nucleus in the ascus, has not yet been studied genetically. No one has yet dissected out and studied in culture this dikaryotic diplont, independent of the enveloping haploid structures. Such a study might be possible with the dikaryophyte of species of *Taphrina* or perhaps with *Asco corticium*, if heterothallic species occur in that genus. In the interest of clarity, students of the genetics of Ascomycetes or Basidiomycetes might well adopt the system of symbols devised by C. E. Allen (1924, 1925) for use in the Bryophytes.

groups. Only a few of these will be discussed further. In the diagrams of Phyllophora an auxilliary cell is indicated. In many of the diplobiontic red Algae there is a fusion of the fertilized carpogonial cell with an auxiliary cell and often there is a multiple fusion with other cells of the carpogonial filament to form a large placental cell from which the gonimoblast develops. Dodge (1929) has compared this situation with the multinucleate fusion cells which have been noted in many rust aecia, and Miss Rice (1933) has found such cells in *Puccinia Sorghi* and has summarized the observations of earlier cytologists dealing with such structures. The evidence is strong that such cells have arisen from a fusion of two or more cells and that a comparison with the red algal auxiliary or placental cells is justified.

The detailed studies of Miss Grubb (1925) on the development of spermatia in a large series of red Algae is strongly suggestive that their method of formation is directly comparable with the development of similar structures in Ascomycetes and the rusts. Indeed Miss Grubb makes such a comparison based primarily on Brooks' (1910) studies of *Gnomonia erythrastroma* and Blackman's (1904) observations on *Gymnosporangium clavariaiforme*. She says "Characters such as these in antheridial structure suggest that there may be something more here than an accidental resemblance to the antheridia of the Florideae." The detailed studies of Brierley (1918) on the spermatia (microcondia) of *Botrytis cinerea*, Drayton (1934) of *Sclerotinia Gladioli*, Dodge (1932) of *Neurospora* and (1936) of *Pleurage anserina*, and Olive (1944) of *Gymnosporangium clavipes* have served to materially strengthen previous evidence as to the essential identity in the method of spermatial formation in the red Algae, Ascomycetes and Uredinales.

The rust basidium as it is exemplified by *Puccinia* and most of the rusts having resting teliospores would seem at first sight to have no homologue in the red Algae. It is the teliospore cell which must be compared with the tetrasporangium as it is here that the fusion of the dikaryon occurs. If, however, we turn to a form such as *Coleosporium* in which both caryogamy and meiosis occur in the teliospore, we do have a situation which may be directly compared with the tetrasporangium, with the added support that many red Algae develop their tetraspores in a "zonate" manner, that is, in a vertical row. The genus *Trichopsora*, properly interpreted as a modified endo-form, is similar to *Coleosporium* in the method of development of the basidia. The genus *Chrysocyclus* (= *Holwayella*) is also of interest in this connection. In the development of the telia of this tropical microcyclic form, upright sporophores cut off two cells at the tip, these continue to elongate, the

lower cell developing to one side and the two forming an elongated "mitten" shaped structure with a broad "thumb." Finally, the contents accumulate in the upper part, and three cross walls form in each of the extended cells. The basidiospores are formed in the usual manner. There appears to be no cessation of development from the sporophore tip to the basidium, and no stage at which a teliospore can be distinguished from the basidium (Sydow, 1925, p. 322; Jackson, 1926). All these genera have waxy sori which fact may be of some significance. The thick-walled resting type of teliospore may have been a development correlated with the overwintering habit or with the necessity to rest during dry periods. Viewed from the background of the genera mentioned the external basidium (promycelium) of the *Puccinia* type may be looked upon as a structure formed as a result of resumption of growth following a rest period.⁴

COMPARISON OF *PHYLLOPHORA BRODIAEI* WITH A MICRO-PUCCINIA

The second comparison of life cycles (Fig. 2) will be between *Phyllophora Brodiaei* and *Puccinia malvacearum*. Both these forms are to be interpreted as microcyclic. To obtain a clear picture of the method of short cycling exemplified by these two forms, attention should be directed again to the diagrams of the long-cycled forms (Fig. 1) and focussed on the position in the diagram of the aecial structure and the gonimoblast and of the ultimate spore forms in each case—the teliospore sorus with its teliospores in the rust diagram, and the nemathecium of tetraspores in the diagram of the red Alga. Short cycling occurs in the rust by the telescoping of the life cycle in such a way that the teliospore sorus replaces the aecial structure (Jackson, 1931). Rosenvinge (1929) and Svedelius (1931) interpret the life cycle of *Phyllophora Brodiaei* in a similar fashion. The tetrasporic nemathecium, a development of the independent diploid plant of the long cycled type, replaces the gonimoblast. As interpreted the tetrasporangia do not simply appear in place of carpospores but the whole diploid nemathecium appears in place of the gonimoblast.⁵

⁴If this viewpoint is correct, then the attempt by Linder (1940) to derive the rust basidium from the ascus by comparison with those *Pyrenomyces* in which an inner wall of the ascus emerges in connection with spore dispersal would seem to have no adequate basis.

⁵The tetrasporic nemathecium of *Phyllophora Brodiaei* was earlier interpreted as a parasite and given the name *Actinococcus subcutaneus*. Other species comparable to *Phyllophora Brodiaei* as to the shortened cycle are now known. Among these *Gymnogongrus Griffithsiae* has a nemathecium which develops both monospores and tetraspores. This nemathecium was also considered a parasite and referred to

The main point of interest here is that, in the two groups, the method of short cycling is the same. In both cases, the diploid phase is reduced and like the gonimoblast or aecial structure which has been replaced, is directly dependent on the haplont. The independent dikaryotic thallus of the rust and the independent tetrasporophyte of the red Alga are dropped out of the cycle. *Puccinia malvacearum* is homothallic (Ashworth, 1931) in the sense that the full cycle is developed from a single

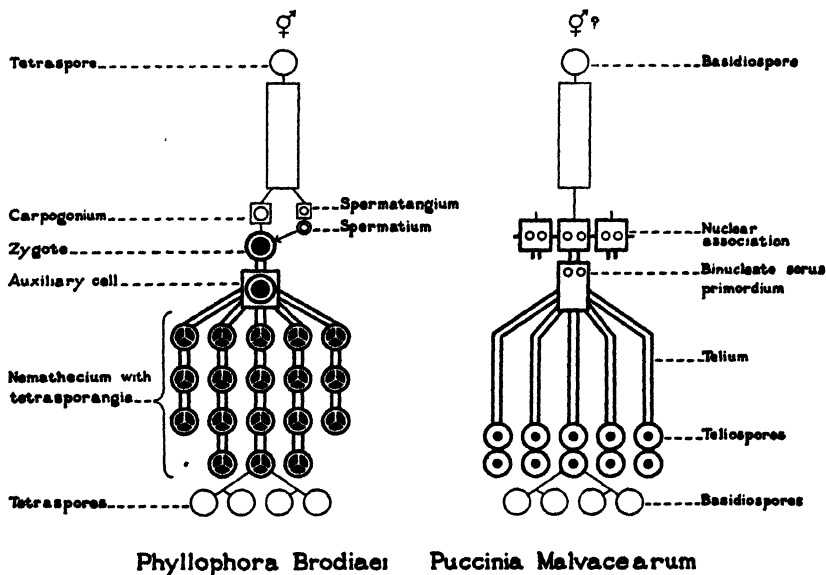


FIGURE 2.—Comparison between a red alga having a shortened cycle, *Phyllophora Brodiaei*, with a typical micro-form of the rusts, *Puccinia malvacearum*.

uninucleate spore and only one thallus is involved. No spermatogonia are present but an association of presumably sister nuclei takes place in a multiple fashion in the sorus plexus. This species illustrates a case of degenerate sexuality; the dikaryon is retained, fusion and meiosis occur in the typical manner—vestigial “habits” derived from the long-cycled parent (Jackson, 1935). *Phyllophora Brodiaei* is sexually

as *Actinococcus aggregatus*. This species has been studied by Gregory (1930, 1934) and Chemin (1933). Another similar form is *Ahnfeltia plicata* which produces only monospores and is presumably reduced to a sexless haplont. It has been studied by Rosenvinge (1931 a and b), Gregory (1930, 1934), and Chemin (1930). It is worthy of note that these genera are all included in the family Phyllophoraceae of the Gigartinales.

homothallic, otherwise the sexual situation would appear to be similar to that of the long-cycled *Phyllophora membranifolia*. A fusion of sex nuclei must occur as Claussen (1929) has shown that meiosis occurs in the tetrasporangia. Though there is no experimental evidence available to substantiate the suggestion, it may well be that in such a case cross-fertility between two hermaphroditic self-sterile haplonts is necessary to ensure fertility as in the case of the long-cycled rust and the Ascomycetes to be discussed later. It might equally be possible that self-fertility together with chance interfertility could be in operation. The cytology of the carpogonial mechanism and of fertilization in this species should ultimately be studied.

Attention may be called at this time to the fact that in *Phyllophora Brodiaei* we have a type of life cycle essentially similar to that of the higher Ascomycetes. I will return to this point later.

COMPARISON OF LIAGORA TETRASPORIFERA WITH AN ENDO-FORM

The third comparison (Fig. 3) will be between the red alga *Liagora tetrasporifera* and the endo-form of life cycle in the rusts as exemplified by *Endophyllum Sempervivi*. The genus *Liagora* belongs in the Nemalionales in which the typical life cycle is haplobiontic, reduction division occurring at the first division of the zygote nucleus and hence the gonimoblast and carpospores are haploid (Nemalion, Batracho-

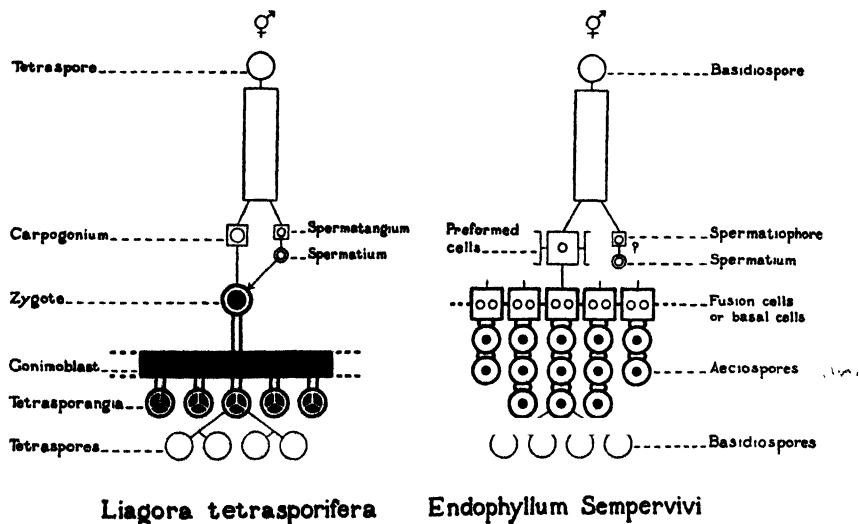


FIGURE 3.—Comparison between the simplified *Liagora tetrasporifera* with an endo-form in the rusts, *Endophyllum Sempervivi*.

spermum Scinaia, etc.). *Liagora tetrasporifera*, described by Børgesen (1927) is apparently unique among the species of this group in that tetrasporangia occur in place of the carpospores on the gonimoblast. While no cytological study has yet been made it is assumed that reduction division has not occurred at the time of development of the zygote nucleus but is delayed and occurs in the tetrasporangia. Under these circumstances the gonimoblast would be diploid. Svedelius (1931) interprets this species as representing a probable intermediate step in the origin of the diplobiontic red Algae from the Nemalion type, meiosis being delayed and occurring in the carposporangia. If reduction should be still further delayed and the carpospores remain diploid, then an independent diploid thallus would result and the diplobiontic type would be realized. This view of the evolution of life-cycle types within the red Algae is the one held by most students of the group.

Another view, discussed by Goebel (1928), and apparently also held by Tilden (1935), is that the diplobiontic type of cycle is the primitive one from which the haplobiontic type is derived by reduction. If this were the case then *Liagora tetrasporifera* would represent an intermediate step in that reduction rather than a step in a progressive evolution as would be the case according to the view first discussed.

Endophyllum Sempervivi, the life cycle of which we are to compare with that of *Liagora tetrasporifera*, is an example of a reduced type of life cycle which, in its typical form, has developed from the long-cycled type by the fusion of the dikaryon occurring in the first spore to appear—the aeciospore—rather than being delayed to the final spore—the teliospore—as is the case in the long-cycled forms. The life cycle is telescoped in the manner suggested and the independent dikaryotic diplont of the parent long-cycled form is dropped. The fusion having occurred,—since reduction follows fusion in all rusts—a four-celled basidium develops on germination of the aeciospore, in the development of which the reduction division occurs. It should be noted, however, that this typical condition is rarely realized in the endo-forms and much variation in nuclear history is found in this group (Jackson, 1931, pp. 48-53). Morphologically *Endophyllum* is an *Aecidium*, the aeciospores having taken over the function of the teliospore because the fusion has been moved backward. The sexuality here may possibly be variable in different races. Usually the species is said not to develop pycnia though deBary (1866) has noted a race in which pycnia developed regularly. Where no pycniospores are developed, the nuclear cycle would be exactly comparable with that of *Puccinia malvacearum* (cf. also Miss Ashworth, 1935 b).

If *Liagora tetrasporifera* represents a step in reduction according to the second view discussed above, then the comparison which I wish to make with the Endophyllum is strengthened.⁶ Both would represent reduced life cycles in which that portion of the diploid phase represented by the gonimoblast of the diplobiontic red alga or the aecial structure of the long-cycled rust is all that is left. Even if the more commonly accepted view of the origin of cycles within the red Algae is considered, the comparison is still valid since the whole diploid phase in both these short-cycled forms represents that portion of the diploid phase of the long-cycled forms which is dependent on the mother haplont. In any case it is worthy of note that we find comparable cases of this type of simplification in both groups. When considered together with the previous comparison in which similar cases of a different type of simplification are found in both groups, some significance may be attached to the phenomena.

⁶The recently reported culture work with members of the family Bonnemaisoniaceae of the Nemalionales by the Feldmanns (1941) may, if their interpretation is corroborated by further cytological studies, prove to be evidence in favour of the minority view that the haplobiontic red Algae are derived from the diplobiontic type by reduction.

Svedelius (1933) has shown that in *Bonnemaisonia asparagoides* and *Asparagopsis armata* reduction is zygotic as in other Nemalionales which have been studied cytologically. The Feldmanns have cultured these same species and found that the carpospores give rise to independent, somatically different sporophytes that produce tetraspores. These sporophytes have previously been classified in the genera Hymenoclonium and Falkenbergia and interpreted as isolated tetrasporic plants of diplobiontic Florideae. Since the carpospores are haploid, the independent sporophytes are assumed to be haploid also. If an independent haploid tetrasporic plant is now present in the life cycle of the species cultured, this could be taken as evidence that this haploid sporophyte originated by simplification from a diploid one and that the species were originally long cycled. The formation of tetrasporangia on such a plant instead of monosporangia could be interpreted as a "habit," now vestigial, developed when the independent plant was diploid and reduction occurred in the tetrasporangia.

Is the tetrasporangium to be derived from the monosporangium or the reverse? In *Gymnogongrus Griffithsiae*, where both types of sporangia occur in the same nemathecium, the evidence seems clear that the reverse is true in that species. This may also be the case in *Ahnfeltia plicata* if Rosenvinge's interpretation of the origin of the nemathecium is the correct one. If the tetrasporangium is phylogenetically the primary structure, then the genus Rhodochorton, as interpreted from the studies of Miss Drew (1928, 1935) provides strong evidence that the diplobiontic cycle is the primitive one in the red Algae. In view of the interpretation by the Feldmanns of the cycle in the Bonnemaisoniaceae, the cytology of Rhodochorton should prove extremely interesting.

COMPARISON OF *AHNFELTIA PLICATA* WITH UNINUCLEATE
MICRO- AND ENDO-FORMS

A fourth comparison, for which no diagram seems necessary, may be made between the red alga *Ahnfeltia plicata* and certain micro- or endo-forms of the rusts. This curious red alga has been studied in considerable detail by Rosenvinge (1931 a), Gregory (1930, 1934) and Chemin (1933). It is a short-cycled form morphologically comparable to either *Phyllophora Brodiaei* or *Liagora tetrasporifera*. The nemathecium, formerly thought to represent a parasite on the *Ahnfeltia* and given the name *Sterrocolax decipiens*, is a wart-like structure composed of parallel filaments which ultimately produce monospores. No sex organs are present though structures which might be interpreted as abortive carpogonia have been observed. It seems evident that the species is maintained through successive generations of sexless haploid plants. Rosenvinge's description of the nemathecium, because of certain structural peculiarities, suggests that in origin it is, as in *Phyllophora Brodiaei*, a product of an independent sporophyte from which it may have been derived, and is apparently so interpreted by Rosenvinge. Svedelius (1937), however, refers to the structure as a gonimoblast. Whichever interpretation is the correct one, similar situations are known in the endo- or micro-forms of the rusts. If the *Ahnfeltia* nemathecium is to be compared with that of *Phyllophora Brodiaei*, then we have a counterpart in the rusts in the short-cycled *Uromyces Rudbeckiae* which is uninucleate throughout (Olive, 1911; Jackson, 1931, p. 23). If this nemathecium is morphologically to be compared with the gonimoblast, then comparable cases occur among those endo-forms of the rusts which have been shown to be uninucleate throughout such as *Endophyllum Centranthi-rubri* (Poirault, 1915), and the uninucleate races of *Gymnoconia nitens* (Dodge and Gaiser, 1926).

Among the diplobiontic red Algae a considerable number of species have been described which are only known from tetrasporic plants. Some of these no doubt represent the independent sporophyte of species for which the gametophytic plant is as yet undiscovered.⁷ Others may represent a simplified type as in *Lomentaria rosea*. In this species Svedelius (1937) has shown that though the chromosome number, as compared with other normal species of the genus, is that expected for the diploid phase, there is no reduction division at the time of tetra-

⁷Still other red Algae in which only tetrasporic plants are known may prove to be haploid sporophytes as in the case of species of *Hymenoclonium* and *Falkenbergia* according to recently reported results of culture work of certain species of *Bonne-maisoniaceae* by the Feldmanns (1941) (cf. footnote 6, p. 17).

spore formation. The species maintains itself through successive generations of diploid individuals because the tetraspores are formed in an apomeiotic manner. The cycle is shortened by the dropping out of the haploid plants. No such type of simplified cycle is known among the rusts. A large number of so-called hemi-forms occur in which only the uredinial and telial stage is known—the independent dikaryophase of the rust cycle. These are for the most part quite certainly to be interpreted as heteroecious rusts in which the aecial phase has not yet been discovered or recognized as belonging in the cycle. It is quite within the range of possibility, however, that a cycle comparable to that of *Lomentaria rosea* may yet be discovered among these hemi-forms. In the short-cycled rust *Puccinia arenariae* (Lindfors, 1924) the whole mycelium is dikaryotic and, viewed superficially, might appear to be comparable. That it is not, is shown by the fact that the dikaryon originates in the basidiospore and the usual fusion occurs in the teliospore. The basidium is two-celled and bears two basidiospores. Each spore receives two of the four nuclei resulting from meiosis. Furthermore, the initial thallus as in all micro-Puccinias, is morphologically that of the haplont (cf. Jackson, 1931).

SUMMARY OF RUST AND RED ALGAL CYCLES

It seems desirable at this point to discuss briefly the evidence which has been presented dealing with the similarity of the cycles as found in present-day red Algae and the Uredinales. In the rusts, there can be little doubt that the heterothallic, heteroecious long-cycled forms are the primitive ones. The essential identity of the unique cycle of the diplobiontic red Algae and the long-cycled rust, particularly the division of the diploid phase into two parts on different thalli, is alone sufficient to suggest the possibility of an origin of the rusts from the ancestors of the diplobiontic red Algae. When to this is added the evidence that, to a remarkable degree, the same sort of life-cycle simplifications and modifications are to be found in both groups, the suggestion of relationship should not be lightly ignored. If all this similarity in cycles is to be interpreted as merely an interesting but not phylogenetically significant series of parallelisms, then those who are satisfied to hold that view must admit that it is a most remarkable series.)

(When to this evidence obtained from similarity of cycles in the two groups we add the essential similarity, though modified in the rust, of the sexual process, the very comparable method of formation of the spermatia together with other comparisons which suggest homologous structures, it becomes even more difficult to deny the possibility of relationship.)

A consideration of the plasmogamic type of life-cycle characteristic of the Hymenomycetes and other groups of the Basidiomycetes might well form a part of the present discussion, but to do so would take more time than is at my disposal. There is no real counterpart to this type of cycle in the red Algae, nor is it likely that one will be encountered in that group. Viewed from a red algal background, it would appear that the extreme simplification in the sexual mechanism which has resulted in the mushroom cycle had developed after the higher fungi had diverged from the algal line.

COMPARISON OF THE RED ALGAL AND ASCOMYCETE CYCLES

We may now turn to the Ascomycetes. This group has often been considered as having originated from the red Algae largely on the basis of the spermatial type of sexuality present in many species and because of the similarity of the morphological structures. Until such simplified red Algae as *Phyllophora Brodiaei* and *Liagora tetrasporifera* were known and properly interpreted, no life cycle comparable to that of the Ascomycetes was known in that group. The relatively recent developments in our knowledge of odd red algal cycles now make it possible to make a direct comparison between the characteristic life cycle of the Ascomycetes with such simplified red Algae.

Briefly stated, the characteristic life cycle of the higher Ascomycetes may be interpreted as one in which the diploid phase (the sporophyte) as in the Uredinales—a dikaryotic diplont—is developed directly from the fertilized ascogonium and remains in association with and parasitically dependent upon the mother haplont (the gametophyte).

The diploid phase of the higher Ascomycete, consisting as it does only of the ascogenous hyphae and the young asci, furnishes little in the way of morphology upon which to judge as to whether this phase is to be compared with the gonimoblast structure of the red alga—as in *Liagora tetrasporifera*—or with a structure which has developed from the independent sporophytic phase and replaced the gonimoblast—as in *Phyllophora Brodiaei*.

For our immediate purpose it matters little whether one considers that *Liagora tetrasporifera*, as interpreted, represents an intermediate step in a progressive evolution from the Nematium type to the Polysiphonia type of life cycle, or that it represents an intermediate regressive step in accordance with the reverse view. Either view as to the evolution of life cycles within the red Algae furnishes the possibility that at some period in the evolution of the group as it now exists there may have been many red Algae having the life-cycle type of *Liagora tetrasporifera*. It is con-

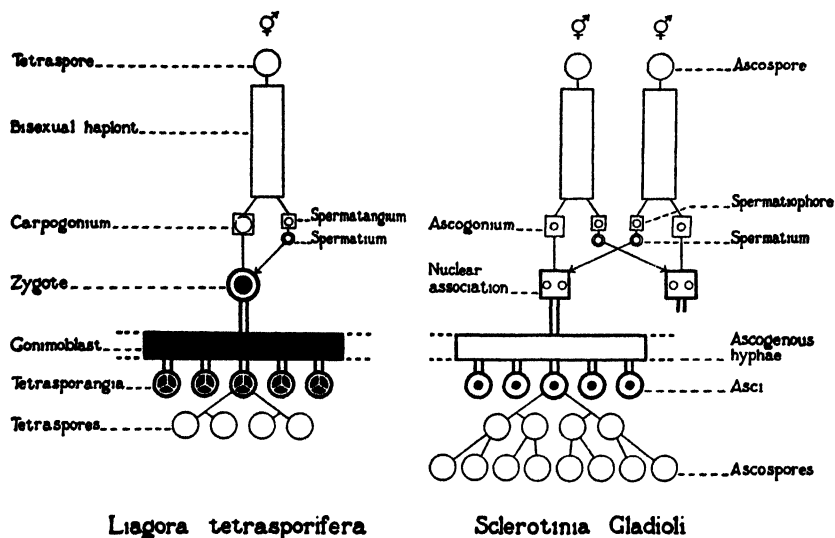


FIGURE 4.—Comparison of the reduced red algal cycle as exemplified by *Liagora tetrasporifera* with a typical Ascomycete having spermatial sexuality, *Sclerotinia Gladioli*.

ceivable that the Ascomycetes may have originated from such forms. There is, however, another possibility. The existence among present-day Algae of such forms as *Phyllophora Brodiaei*, *Gymnogongrus Griffithsi*, and perhaps also *Ahnfeltia plicata*, suggests that at some remote period in the evolution of the group the tendency to "short cycle" in the manner exemplified by *Phyllophora Brodiaei* may have been much more evident than it is today and short-cycled forms of this type may have occurred abundantly. Such a situation would also provide a theoretical starting point for the Ascomycetes in so far as the life cycle is concerned.

The suggestions in the preceding paragraph would provide for a polyphyletic origin of the Ascomycetes, if such is needed, direct from the red Algae. As will be pointed out later, however, the problem of the dikaryon, its origin, and the fact that this association of sex nuclei is universal also in the Basidiomycetes, makes such a polyphyletic origin difficult and perhaps quite unlikely. If Algae comparable to the odd *Liagora* or the *Phyllophora* had been abundant in the past, it would seem there should be more evidence of such relic forms among present-day red Algae than appears to be the case, though further studies may show that they are much more abundant than has been suspected. For our purpose it is the existence of such life-cycle types among the red Algae that is of paramount interest.

If a choice of the two life-cycle types in the Algae must be made, it would seem, from the evidence to be obtained from comparisons of structures in existing forms of both groups, particularly the resemblance of the red algal cystocarp to the ascocarp of some Pyrenomycetes, and Discomycetes, that the Ascomycete sporophyte is most likely to have been derived from the gonimoblast and hence the life cycle as exemplified by *Liagora tetrasporifera* offers the better comparison.

For purposes of direct comparison of the life cycles, two diagrams are provided (Figs. 4 and 5) in which a typical Ascomycete is compared

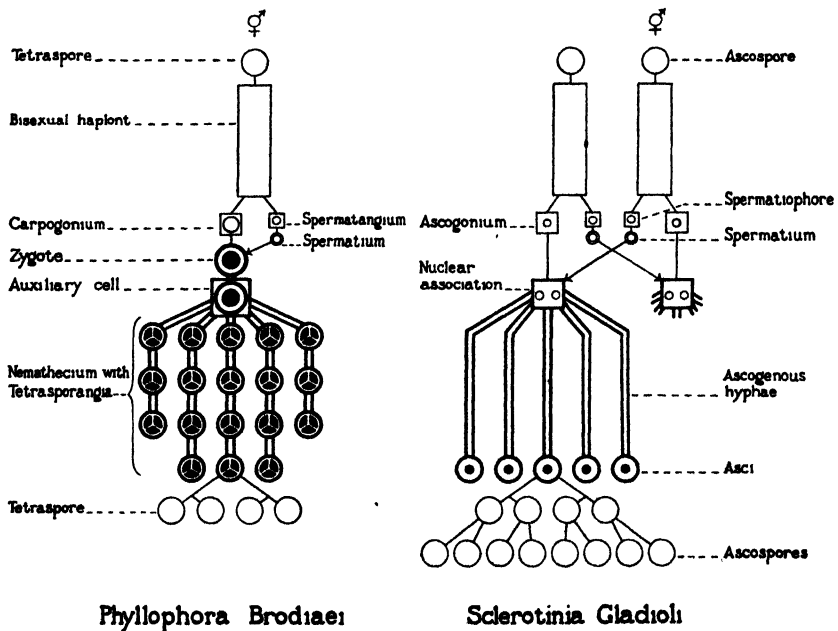


FIGURE 5.—Comparison of the Ascomycete cycle with that of *Phyllophora Brodiaei* interpreted as a reduced cycle in which the gonimoblast has been replaced by the tetrasporic nemathecium.

first with *Liagora tetrasporifera* and then with *Phyllophora Brodiaei*. The Ascomycete diagrams are varied in the two figures in order to bring out as clearly as possible the comparison with the two different red algal cycles. For the Ascomycete, *Sclerotinia Gladioli* (Drayton, 1934) is selected largely because in this form spermatial fertilization appears to be obligatory. The heterothallic species of *Neurospora* or the heterothallic phase of *Pleuraea anserina* would answer the purpose as well were it not for the complication of modifications or substitute processes which

may take the place of spermatial fertilization. The diagram would be the same for such forms if only the spermatial method of bringing the sex nuclei together were considered.

In *Sclerotinia Gladioli* two bisexual haplonts are involved in sexuality. Spermatia (microconidia), as well as receptive columnar outgrowths, which develop from stromatic tissue, are developed on every haploid thallus. In order to obtain the development of apothecia, asci, and spores, the spermatia must be placed on the receptive columnar structures. Though cytological studies are as yet incomplete, Drayton has shown that a coiled ascogonium is present in the tissue of the columnar outgrowths and there is evidence that the trichogyne extends to the apex of this structure and there receives the spermatial nucleus, though this detail has not yet been observed. At any rate there is no further development of these columnar structures, which are to be interpreted as apothecial initials, unless spermatia from another compatible haploid thallus are placed in position to be contacted by the trichogynes. Following spermatization the columnar structure begins to elongate, ascogenous hyphae develop from one or more of the cells of the coiled ascogonium, make their way through the haploid tissue of the developing apothecial initial, and finally develop asci which are interpolated between the paraphyses at the upper surface of the apothecium.

As in the Uredinales, the two sex nuclei, one derived from the ascogonium and the other from the spermatium, associate but do not unite in the ascogonium.⁸ They divide in association throughout the development of the ascogenous hyphae and finally fuse in the young ascus where the fusion nucleus immediately undergoes meiosis. An additional mitotic division results in eight nuclei around which the eight ascus spores are differentiated. The diplont of the Ascomycete, as in the Uredinales, is here interpreted as a dikaryotic diplont. The fusion in the ascus represents a delayed sexual fusion.

Here again possible homologues may be inferred from the diagrams. It is not my purpose to discuss these in any detail. This phase has been admirably covered by Dodge (1914) and E. A. Bessey (1942), with detailed comparisons of morphological structures in the two groups as well as with the ascomycetous Lichens.

⁸It is necessary at this point to choose between conflicting theories as to the Ascomycete dikaryon, and to discard the principle of double fusion and brachymeiosis as of doubtful validity and quite certainly not of universal application in the Ascomycetes. I find myself in full agreement with the statement headed "Double fertilization in Ascomycetes" in Dodge's (1939) address presented at the Third International Congress for Microbiology.

At the time Dodge wrote, only observational evidence dealing with the Ascomycetes having a spermatial type of sexuality was available. Since that time the epoch-making experimental studies of Dodge and others on sexuality in *Neurospora*, Drayton in *Sclerotinia*, and Ames in *Pleuroge* have proven that spermatia in these Ascomycetes are functional as male cells in fertilizing the ascogonium and are received by the trichogyne of the latter or by other receptive hyphae which develop therefrom (Backus, 1939). A careful analysis of the studies that have been made on the genera mentioned strengthens materially previous concepts of a relation between Ascomycetes and red Algae. The essential identity of the method of spermatial formation in these genera and in the red Algae has already been mentioned (p. 12).

It will have been noted that in attempting a derivation of the Ascomycetes from the red algal line, it is the higher forms having a spermatial type of sexuality that are assumed to be the primitive ones or at least to show the closest comparison. This viewpoint requires the derivation of the Ascomycete antheridium from the spermatium. Dodge (1914) has quite successfully shown how this may be accomplished, using evidence from the ascomycetous Lichens and from certain species of *Ascobolus*. It seems unnecessary to review this evidence at this time. So far no mention has been made of the Laboulbeniales which have long been recognized as showing evidence of relationship with red Algae. The closest comparison with the red algal trichogyne and the method of formation of the spermatia is to be found in this group. Miss Grubb (1925), in connection with her detailed study of red algal spermatangia, comments on this close comparison.

This method of approach to the search for the ancestors of the Ascomycetes also requires the assumption that the simpler forms in this group are not the primitive ones but have been derived through reduction. This phase of the subject has been well covered by Bessey (1942) and the right-hand part of his Fig. 5 is reasonably acceptable as a family tree for the lower Ascomycetes.

It would be desirable, if time permitted, to review the various modifications of the basic life-cycle type of the higher Ascomycetes, and the variations in the sexual mechanism which are known to occur. In the interest of brevity, it will suffice to mention that all sorts of modifications of the sexual process appear to have taken place. Homothallism, both of the "sexual" and "self-fertile" types, is common and has resulted in loss or abortion of sexual structures. Perhaps the most significant trend in a modified sexuality is that exemplified by the heterothallic phase of *Gelasinospora tetrasperma* (Dowding and Buller, 1940) and the hetero-

thallic *Humaria granulosa* (Gwynne-Vaughan and Williamson, 1930), where a form of plasmogamy has replaced the normal method of bringing the sex nuclei into association. In *Gelasinospora tetrasperma* spermatia are not present and in *Humaria granulosa* the antheridia are absent. Vegetative nuclei from compatible haplonts serve to fertilize the ascogonia by means of hyphal fusions and nuclear migration. It seems probable that this sort of simplification may be very common in the higher Ascomycetes in those cases where no male structure is evident. Such a method might be expected to result in a simplification of the ascogonium, as in *Humaria granulosa* where the trichogyne has disappeared. Many Ascomycetes in which only abortive "Woronin hyphae" are known may prove to be of this type.

GENERAL DISCUSSION

It is to be expected that many difficulties arise in connection with an attempt to derive the two great groups of the higher Fungi from red algal ancestral forms. These difficulties, however, seem no greater than the problems encountered when the Fungi are viewed as one monophyletic series. Some of the problems are the same, whichever viewpoint is taken, particularly those dealing with the relationship of Ascomycetes and higher Basidiomycetes. It is not within the scope of the present contribution to attempt any detailed discussion or to go very far in suggesting possible solutions of these difficulties. It would seem, however, that a general statement concerning some of the more difficult problems which are encountered is desirable and that an attempt should be made to develop an hypothesis which will, on theoretical grounds at least, take care of the main difficulties.

In the comparative consideration of the Uredinales and Ascomycetes, I have so far treated the subject as though both these groups might be derived directly from the red algal line without reference to each other. This raises a very fundamental question which must be briefly considered. If an origin of the higher Fungi is to be sought from the red Algae, has this been a monophyletic or a polyphyletic one? The rusts show the closest comparison as to life cycles and the evidence is strong for a direct origin, particularly so if the diplobiontic red Algae are considered the primitive ones in the group (Orton, 1927). Can the Ascomycetes be derived from the rust line? Most authors who have considered this question have taken the opposite view and would attempt a derivation of the Uredinales from the Ascomycetes (C. E. Bessey, 1894; E. A. Bessey, 1913, 1942; and Linder, 1940) but one of the chief difficulties in such a view lies in the fact that the Ascomycetes cycle is a short cycle and is

perhaps also a simplified one. If the Ascomycete cycle is considered to be derived and to represent a regressive step in the evolution from the diplobiontic red Algae, a view which I have chosen to take,⁹ then a reversal to the original type of cycle is not to be expected as would be required if the rusts are to be derived from the Ascomycetes.

Another difficulty centres in the time of origin of the dikaryon, the ascus hook, and the Basidiomycete clamp. The dikaryotic diplont represents a unique association of sex nuclei and appears to be universal in the higher Ascomycetes and in the Basidiomycetes. It is unlikely that the dikaryon has arisen more than once in the evolution of these groups. If the explanation that the dikaryon has arisen simply by a delay in the fusion of sex nuclei can be generally accepted, then it would seem reasonable that this modification in the nuclear cycle came very early, and the hook and clamp came simultaneously or later as a mechanism to ensure that the two sex nuclei were kept in pairs for ultimate fusion in the ascus or basidium. I cannot join forces with those who would deny that the ascus hook and basidium clamp are homologous structures. Rogers' (1936) study of the development of the clamp in connection with basidial formation in *Sebacina* is convincing that they must be so considered. It seems unlikely that such characteristic structures have developed independently in the evolution of the Ascomycetes and Basidiomycetes.

One is tempted to agree with Linder (1940) that the Basidiomycetes can all be derived through the rust line, though his attempt to derive the septate basidium from the ascus is not convincing. The parasitic Auriculariaceae, including such forms as *Septobasidium*, *Uredinella*, *Eocronartium*, *Herpobasidium*, *Jola*, and the basidial phase of *Glomerularia Lonicerae* can be considered very close to the rusts and quite certainly derived directly from that group. As is the case in the rusts, none of these genera bears clamps on the mycelium. *Septobasidium*, *Uredinella*, *Herpobasidium*, and an undescribed *Jola* (?) on ferns all have coiled haustoria comparable to those of the rust genus *Milesia*. If one goes much farther in admitting close relationship between the rusts and the Auriculariaceae, the presence of clamps is encountered (Martin, 1942). The clamp-bearing members of this group are for the most part saprophytic. The clamp is not a structure

⁹The other alternative is to consider that the Ascomycetes are to be derived from a life-cycle type as represented by *Liagora tetrasporifera* when that form is interpreted as representing a progressive step in the evolution of the red algal cycles. This would either require an independent origin of the Uredinales from the diplobiontic cycle or the rust cycle must be derived by amplification from the Ascomycete cycle. While this view may ultimately need to be considered, I have for various reasons chosen to accept the minority view as to relationship within the red Algae.

that would be expected to be lost and then to reappear, and on this account a direct origin of all the Auriculariaceae and other Tremellales together with the Hymenomycetes from the rust line presents a real difficulty. Did the rusts at some stage in their evolution possess clamps or did they have their origin in an algal group in which the dikaryon and the progenitor of the clamp had already been established? If so, can the remainder of the Basidiomycetes be derived from an early stage in the evolution of the rust line when clamps may have been present?

If the Basidiomycetes are derived from the Ascomycetes, as would be compulsory under the monophyletic view of fungus origin, then the basidium must be derived from the ascus. If these groups are to be derived from red algal ancestors, such an origin of the basidium is not necessarily required. The ascus and the basidium, in such a view, would both be homologous with the tetrasporangium and might have been derived independently from that structure.

The difficulties which have been discussed represent only a part of the problems encountered but are the ones which seem most fundamental and must be considered in any theory dealing with the derivation of these groups from holophytic ancestors. Our discussion would not be complete without an attempt at the formulation of a workable theory.

A TENTATIVE THEORY

Viewed, then, from the background which has been chosen for consideration today, a tentative speculative concept such as follows may be suggested which will serve to minimize the difficulties which have been raised. At some remote period in the evolution of the red Algae, in a diplobiontic group, the sexual mechanism may have become modified in such a way that the sex nuclei came to be associated in the carpogonium but did not unite until later, just previous to meiosis, in the tetrasporangium—thus accounting for the origin of the dikaryon. Accompanying this sexual modification a mechanism developed which ensured keeping the sex nuclei in pairs—the forerunner of the ascus crozier and the Basidiomycete clamp. From this basic algal group it may be postulated that three groups evolved all of them ultimately developing to “land” plants. Two of these groups evolved as a reduction of the life cycle in the direction of a dikaryotic gonimoblast. One developed to the Ascomycetes in which the crozier was retained and the tetrasporangium developed to the ascus. In the evolution of the ascus from the tetrasporangium the sporangial habit of spore formation was retained, and as the group emerged to a “land” habitat, a special method of air dispersal of the spores was evolved. The other short-cycled group developed to the Hymenomycetes and other related groups. In this line the clamp

was retained, the sexual mechanism became altered early to a plasmodic type of sexuality; the tetrasporangium, perhaps originally forming its spores in a "zonate" manner, became modified to the basidium, in the development of which a method of air dispersal which involved the migration of the nuclei into externally developed spores was evolved. This basic basidium may have been of the *Auricularia* type from which the other heterobasidia may have been derived, finally culminating in the homobasidium (Linder, 1940). The third group, retaining the long cycle, developed to the *Uredinales*. Perhaps for a time clamps were present but were ultimately lost. It may well be that the Basidiomycete line referred to above emerged from the rust line, as a simplified cycle, before the clamp was lost. From the tetrasporangium, which was of the "zonate" type, the promycelial type of basidium gradually evolved.

It seems probable that this hypothetical basic red algal group from which the higher Fungi are to be derived was parasitic (Orton, 1927). Perhaps the origin of the dikaryon may have been a result of the parasitic habit. The *Uredinales* have remained obligately parasitic and for the most part retained the long cycle, though showing a tendency to simplification by reduction in various ways. From this line various other parasitic forms have evolved including *Septobasidium*, etc., which have had a remote but probably more recent origin than the other *Auriculariaceae*. It is worthy of note that the species of *Septobasidium* and *Uredinella* are parasitic on insects, while those of *Eocronartium*, *Herporbasidium*, and *Jola* are all parasitic on mosses or ferns. The line which has culminated in the *Hymenomycetes* and related forms became, for the most part, saprophytic. The *Ascomycete* line, while probably for a long time composed of parasites, now includes many saprophytic forms and facultative parasites. The obligate parasitic tendency would seem to be on the way out in the *Ascomycetes*, leaving however several such relic groups.

CONCLUDING STATEMENT

If some definite conclusion or statement of firm conviction is considered essential as a reward for your patience or as a penalty imposed upon your speaker for having involved himself in such a speculative and controversial subject, I shall probably disappoint you. I am, however, willing to make the following concluding statement. In view of the present relatively incomplete state of knowledge of the higher Fungi and of the red Algae, but with the certain promise that our field of vision will inevitably be increased by new discoveries, it is my considered opinion that to dismiss the ancestral red Algae from consideration as a possible starting point for the origin of *Ascomycetes* and *Basidiomycetes* is premature. }

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NEMATOPHORES IN AMERICAN HYDROIDS

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IN hydroids, as in other coelenterates, the nematocyst is a characteristic feature. The structure, of itself, is not cellular but rather it is the product of a special type of cell, known as a cnidoblast, in which it is wholly contained before discharge.

It consists of a capsule containing a coiled tube that is fastened to one end of the capsule and is there continuous with the capsule wall. The capsule may be elliptical, pyriform, oval, or spherical. The attached end of the tube is covered by an operculum. From the free surface of the cnidoblast, near the top of the nematocyst, a bristle, the cnidocil, projects. It is set in a depression of the surface around which the surface is elevated, and is, at times, encircled by stiff rods.

The nematocyst tubes vary much in length and in diameter, and may be of similar diameter throughout, or different in different parts of the length. In all the hydroid nematocysts, the tube is thorny throughout either a portion, or the whole of its length.

At discharge, the operculum springs open and the tube everts, turning inside out in doing so, the tube thus becoming continuous with the capsule wall with which it retains contact. The discharge is associated with a reaction in the cnidocil, but whether the association is entirely or partially mechanical, physical, or chemical, has not been fully elucidated. Some doubt exists as to whether the reaction in the cnidocil is always necessary to produce a discharge.

The nature and size of the capsule and of the tube vary much in different nematocysts, so that Weill has recognized and named 17 different kinds present in the whole phylum, but few of these are found in any one species, genus or family. In most of the types, the tube is open at the end when it is everted and through this opening the capsular contents are ejected. The nature of the ejected material has not been determined definitely and it is quite probable that it is different in different types of nematocysts, or even in different species, but evidently, in most cases if not in all, it is to some degree toxic. In some types the penetrating power of the ejected tube is strong enough to pass for some distance into soft tissues, so that the ejected material is free to cause paralysis or even death of the small animal attacked, and thus the organism can be carried to the mouth with little or no resistance.

Quite commonly a cnidoblast produces but one nematocyst but in other cases each may produce several of them to form a battery, in which case, different types of nematocysts may be represented.

Most of the cnidoblasts are situated where they have the greatest chance to be of use, viz., on the tentacles of the hydranth, where they often appear in clumps or in whorls around the tentacle, but they may appear on any exposed surface. In the *Gymnoblasteria* this grouping is especially evident in families such as the *Corynidae*, where the tentacles are capitate, and in such polymorphic genera as *Hydractinia*, where the defensive zooid has a terminal swelling made up to a large extent by nematocyst batteries.

In the genus *Balella* (Balea), Family *Tubidendridae*, with only one American species reported, *B. irregularis*, and this from the Galapagos islands, there is a structure developed, unique among the *Gymnoblasteria*, or among all of the hydroids for that matter.

The stem of *B. irregularis* is fascicled, and the separate tubes are provided with perisarc. There are scattered foramina through the perisarc, but the perisarc around the foramina is not modified or elevated. Through these foramina project unprotected, coenosarcial processes that look like short tentacles, well provided with nematocysts. They serve as the nearest approach to the calyptoblastic sarcostyles to be found among the American *Gymnoblasteria*. There is no indication, however, that the *Calyptoblastea* are descended from the *Tubidendridae*, or anywhere near that family.

In the *Calyptoblastea*, except for the nematocysts on the tentacles, they are not so widely distributed as in the *Gymnoblasteria* since most of the surface is protected by impenetrable perisarc, but in several of the genera and in the whole family *Plumularidae* special provision is made for the production and possible discharge of groups of nematocysts from the stolon, the stem, and regular or modified branches, primary or secondary. At definite positions, groups of cnidoblasts are developed, commonly raised on papillae or stalks. In order that the threads or tubes may pass to the outside, foramina appear in the perisarcial wall through which the tubes may be exerted. Commonly the perisarc is built up around the foramen to form a theca or cup that serves for protection for the nematocysts similar in nature to the hydrothecae or gonangia.

The group of nematocysts with the supporting stalk or papilla is called the sarcostyle or nematophore, whereas the theca is called the sarcotheca or nematotheca. In taxonomic descriptions, the term "nematophore" is commonly used to refer to the whole structure, sarcostyle and sarcotheca, and it is in that sense that it is used in this paper.

As a matter of fact, in preserved specimens, the sarcotheca is often in good condition when the sarcostyle has disintegrated so that the sarcotheca is commonly described without reference to the sarcostyle, but it is still spoken of as a "nematophore." This meaning is given to the term when nematophores are said to be movable or eleuthero-plean when they are articulated with the basal perisarc, and fixed or statoplean, when there is no such articulation.

In some cases, the perisarc is simply elevated to form a papilla, through a foramen in the tip of which the discharge tubes may be exerted. Such a structure is known as a perforated process or a pseudonematophore. When the nematophore is more highly developed the cavity of the nematotheca may form a single chamber (monothalamic), or the chamber may be divided by a partition to form two chambers (bithalamic).

Although these nematophores are small, they are usually quite conspicuous, and hence they have been used as characters differentiating genera. It is possible that their importance as such has been overstressed. In one family, the *Plumularidae*, all of the species of all of the genera are provided with nematophores but that is not so in any other family as it is now constituted. In some cases where species are placed in different genera according to the presence or absence of nematophores, two species belonging to different genera may have greater similarity apart from this feature than there is between either of these species and other species in the same genus.

With the possible exception of the one family where these structures are found throughout, there appears to be no parallelism in the development of nematophores and in the evolution of other characters. It looks much more as if the development in the genera has been sporadic. Hence it may be that any classification of genera on the basis of the presence or absence of nematophores lacks full genetic support. On the other hand, there is little or no evidence that the development is entirely or even mainly ecological. It may take extensive experimental investigation to settle the question.

To discuss the question more in detail all North American species of hydroids and those from the Pacific coast of South America as far south as Port San Juan, Peru, are taken into consideration.

Nematophores appear in 4 families. In the *Plumularidae* all the genera are provided with them; in the 3 others, only a portion of the genera. In the *Campanulinidae* nematophores appear in *Lafoeina*, *Egmundella*, and *Oplorhiza*; in the *Halecidae*, in *Ophiodissa* only; in the *Lafoeidae*, in *Eucryptolaria*, *Lictorella*, and *Zygophylax*.

It may be well to take up first the genera in which the nematophores seem more or less incidental, leaving those in the family *Plumularidae* for fuller discussion later.

In the *Campanulinidae* each of the genera in which nematophores are present is well paired up with corresponding genera without nematophores: *Egmundella* with *Lovenella*, *Oplorhiza* with *Eucuspidea*, and *Lafoeina* with *Cuspidea*, although in the last pair the resemblance is much less marked. In each case it looks as though the nematophorous genus has been derived from the non-nematophorous genus. There is no similar resemblance in any two of the nematophorous genera.

The nematophores are much alike in *Egmundella* and *Oplorhiza*. They resemble buds given off from the stem or stolon, without articulation, narrowed proximally to form a stalk, leaving a distal portion oval or orbicular in shape, the distal end being rounded, the circular aperture occupying much of the end. There is a difference in size and relative diameter in the different species. In *E. fasciculata*, *E. gracilis*, and *E. grandis*, the distal portion is orbicular with a diameter much greater than that of the stalk, decreasing in size in the order given, those in *E. grandis* being quite minute, 0.05 mm. in diameter. In *E. gracilis* and *E. grandis* they appear on the pedicel or on the stolon; in *E. fasciculata*, on the pedicel or on the tubes of the fascicle. In *E. superba*, the distal portion is more elongated and more slender, not much greater in diameter than that of the stalk. The nematophores grow from the stolon only.

In the single species of *Oplorhiza*, *O. parvula*, the nematophores are somewhat of a compromise between the spherical chambers of the 3 species of *Egmundella* and the scarcely enlarged chamber of *E. superba*. The chamber is oval, elongated to be greater in length than the stalk, much larger than any of those in any species of *Egmundella*. They appear on the stolon only.

The genus *Lafoeina* appears in a niche separated from the remainder of the campanulinid genera. If it is to be compared with *Cuspidea*, apparently its nearest relative, it would seem that the stolon had become fascicled—an unusual condition—and later became erect, for the *Cuspidea*-like hydrothecae are attached to the main tube, as in a common type of fascicled stem.

The nematophores are just as unique for they are shaped like the long hydrothecae, almost as long as they are but much more slender; they may be bulbous at the base. They are developed from the peripheral tubes. The aperture is laterally placed just at the distal end. In *L. maxima* the nematophore is of much the same diameter throughout,

in *L. claviformis*, the terminal portion is enlarged so that the nematophore is clavate.

In 2 of the campanulinid genera, therefore, the nematophores are small and primitive, while in the other genus they are very large with little likeness to other nematophores anywhere.

In the *Halecidae* there is one genus supplied with nematophores. Except for the presence of these structures the species of *Ophiodissa* might well be included in the genus *Halecium*.

The nematophores of the 7 species are all of the same type; tubular, articulated with the basal perisarc, monothalamic. The smallest structures appear in *O. gracilis*, where they are quite minute, and they increase in size through *O. carchesium*, *O. laxa*, *O. negligens*, *O. alternata*, *O. caciniiformis*, and *O. corrugata*, although in some cases the increase is slight. In *O. caciniiformis* and in *O. corrugata*, the margin is flaring but it is not so in any of the others.

Their position varies greatly in the different species. In *O. caciniiformis* and *O. gracilis*, they appear on the stolon, the stem and the pedicels; in *O. laxa* and *O. negligens* they appear on the stem and the pedicels but not on the stolon; in *O. corrugata*, on the stolon and on the stem but not on the pedicels; in *O. alternata* on the pedicels only; and in *O. carchesium*, on the stolon only.

In the *Lafoeidae*, each of the genera in which nematophores are present is well paired with a corresponding genus without nematophores: *Eucryptolaria* with *Acryptolaria*, *Lictorella* with *Lafoea*, and *Zygophylax* with *Grammaria*. There is little difference in the nematophores in the 3 genera; they are all small, articulated to the basal perisarc, monothalamic, tubular.

In the single species of *Eucryptolaria*, *E. pinnata*, the nematophores appear at the base of the hydrothecae but also scattered over the peripheral tubes in the fascicle.

In *Lictorella*, the position varies in the different species. In *L. carolina* and *L. convallaria*, they appear in the axils of the pedicels; in *L. crassicaulis*, they appear in some cases at the base of the pedicels but more definitely on the basal portion of each branch; in *L. crassithecæ*, they have been observed only on the tubes of the fascicle. In Clarke's original description of *L. geniculata* he neither describes nor figures any nematophores, but it is very probable that they are present.

In the different species of *Zygophylax* the nematophores are similar and similarly placed. In the 3 species, *Z. adhaerens*, *Z. chazalici*, and *Z. rigida*, they appear on the short pedicel; in the last of these they

do not appear elsewhere, but in the other 2 species they appear also on the peripheral tubes.

Each of the nematophorous genera in these families has a counterpart among the non-nematophorous genera, and occasionally this is true of the species also. All the evidence available points to the probability that the nematophorous genus is derived directly from the non-nematophorous counterpart and not from any nematophorous genus. While the campanulinid nematophores are more primitive than the others, there is no constant generic difference between the lafoeid and the halecid nematophores.

This all adds up to the conclusion that, in so far as these 3 families are concerned, there is no regular evolution in the nematophores in any way corresponding to the evolution of the families, genera, and species, in which the nematophores appear.

In the large family *Plumularidae*, the nematophores attain to a more important status. Nematophores appear in every genus and species in the family, and there is enough variety in their nature, size, and position, to make them useful as aids to diagnosis of genera and species. They may be fixed or movable or simple perforated processes (pseudo-nematophores); they may be monothalamic or bithalamic; they may be tubular, spurlike, campanulate, triangular, or papilionaceous; the aperture may be terminal or lateral or both; the margin may be entire, serrate, or serrulate; they may appear on the stem and branches, on the hydrocladia, and on the phylactogonia or corbulae.

Because of the apparent importance of mobility or lack of it in plumularian hydroids, the family is commonly divided into 2 portions; the Eleutheroplea, in which the nematophores are movable, and the Statoplea, in which they are fixed. This division fits in very well with differentiation in other characters and it leaves but one intergrading American genus in which both types of nematophores appear.

The eleutheroplean nematophores are simple in their makeup so that they provide little diagnostic variety except in their position in the colony. They are not much more highly developed than those in the *Lafoeidae* and the *Halecidae*, except that they have two chambers rather than one. There is little or no stalk but the proximal chamber is very narrow at the base; it gradually increases in diameter and the increase continues into the distal chamber, so that the whole nematotheca is campanulate, or wedge-shaped in vertical section. The walls are smooth and regular, the margin is entire, and the aperture is terminal, occupying the whole of the end. The cauline nematophores are similar in structure

and size to the hydrocladial nematophores and to the gonangial nematophores when these are present.

The cauline nematophores appear either in the axil of the hydrocladial process or on the free portion of the internode. Some of the hydrocladial nematophores are paired and hence to some extent lateral, while others appear singly and are medially placed. In the rare cases where there are gonangial nematophores, they appear in one pair or in two pairs on the face of the gongangium near its base.

In the genus *Plumularia*, there is little variety in the position of the cauline nematophores. In most species there is one nematophore in the axil of the hydrocladial process or near it; in a much smaller number, there are 2 such nematophores; only in 3 species, *P. altitheca*, *P. magellanica*, and *P. paucinema*, are they absent. In many species there is but one internodal nematophore; in a few, there are 2; in the *P. catharina* group, there are 3, or 2-4; in *P. tenuissima*, there are 4, and in nearly all the species with fascicled stems they are numerous. In 7 species, these internodal nematophores are absent.

As to the hydrocladial nematophores, nearly all the species have a pair placed either above or lateral to the hydrotheca. The exceptions are *P. paucinema*, where there is one very small nematophore above the hydrotheca, and *P. altitheca*, *P. inermis*, and *P. magellanica*, which have no such nematophores.

On the thecate internode, there is usually one mesial nematophore; 5 species have 2 or 1-2 such nematophores; 2 species *P. paucinoda* and *P. polynema* have 3; *P. magellanica* is the only species in which they are absent.

Where athecate internodes are present in the hydrocladium, most commonly there is one mesial nematophore on each except the proximal, which is in most cases free of them; in 5 species there are 2 nematophores on each athecate internode with the exception of the proximal, which may have one or none; in some species of the *P. catharina* group, there may be as many as 3; in 5 species they are absent.

In the genus *Calvinia*, with the single species, *C. mirabilis*, the cauline nematophores have a similar arrangement to that in the species of *Plumularia* that have fascicled stems, but because of the special feature in the hydrocladium, the nematophores here take up new positions.

In place of the mesial nematophore that commonly appears immediate-proximal to the hydrotheca, there is a process like the hydrocladial process of the cauline internode in *Plumularia* that gives rise to a three-jointed spur that tapers to a point; the hydrotheca is situated

in the axil of this process. There are 2 supracalycine nematophores and 1 between the hydrotheca and the process; 2 mesial, on the internode, proximal to the process, and usually 1 on each joint of the spur.

In the genus *Antennella*, since there is no erect stem, there are no cauline nematophores. The hydrocladial nematophores are similar in position to those in *Plumularia*, but with some distinct differences. In all species there are thecate and athecate internodes. On the thecate internode, there is always a pair of nematophores related to the hydrotheca, but in some species, e.g., *A. secundaria*, or *A. quadriaurita*, they are definitely lateral, while in others, especially in *A. curvitheca* they are definitely supracalycine, separated by a short space, at the middle line. On each thecate internode, there is at least 1 mesial nematophore proximal to the hydrotheca; in *A. curvitheca* there are 2. Distal to the hydrotheca, there is at least 1 nematophore but in some species it is so much reduced that it is little more than an opening in the perisarc, a very primitive pseudonematophore, through which the nematocysts may be discharged. In *A. secundaria*, *A. avalonia*, and *A. compacta*, this reduced nematophore is in the angle between the hydrotheca and the internode; in *A. gracilis* it is shaped like the typical nematophore but is smaller and is some distance distal to the hydrotheca; in *A. curvitheca* it is almost as large as the others, and is placed near the distal end of the internode; in *A. quadriaurita*, there are 2 smaller nematophores, forming a pair, each growing out from the base of the pedicel of one of the lateral pair, almost at right angles. On each athecate internode there is but 1 mesial nematophore, except in *A. curvitheca*, where there are 2. In every case where the gonangium has been observed, there is a pair of nematophores near the base.

In the genus *Monostacchas*, with only one American species, *M. quadridens*, the stem is branched and the hydrocladia arise from the branches only. There are no nematophores on the stem but on each internode of the branches there are 3-6; none in the hydrocladial axil. In the hydrocladium, there are alternating athecate and thecate internodes on which are nematophores similar and similarly placed to those on some species of *Plumularia*. On the thecate internode, there are usually 2 mesial nematophores. There is a pair of nematophores near the base of the gonangium, similar to the pair in species of *Antennella*.

In the genus *Antennopsis* there is not much variation in the nematophores. There is at least 1 cauline nematophore in the axil of each hydrocladial process, and in *A. longicorne* and *A. nigra* there are 2; in *A. longicorne*, where the process is long, there may be 1 near the distal end of the process, and in *A. nigra* there is a pseudonematophore

on the basal portion of the process. There are no other cauline nematophores except in *A. distans*, where there may be 1 on each internode. Each species has typical supracalcine nematophores; in *A. hippuris*, there is a mesial nematophore proximal to, and one distal to the hydrotheca; all the other species have only the first of these. On the athecate internode, there are 2 nematophores in *A. distans* and *A. hippuris*, and only 1 on this in each of the other species.

In the genus *Antennularia*, there is comparatively little variation in the nature, position, and number of the nematophores. Of the cauline nematophores, in the placing of them on the hydrocladial process, the species are almost equally divided into those with no regular nematophores, those with 1 nematophore, usually in the axil, and those with 2, either both in the axil or near it, or 1 placed proximally and the other distally. In all but 2 species, *A. simplex* and *A. verticillata*, there is a pseudonematophore present on the process as well. On the stem between the processes, there is more variation. In several species there is none, in others 1, and in others they are varied and scattered.

On the hydrocladial nematophores, every species has a supracalcine pair. On the thecate internode, there is usually but 1 mesial nematophore, but occasionally there are 2: in *A. hippuris*, these are both proximal to the hydrotheca but in the other species there is 1 proximal and 1 distal. Where there are athecate internodes present, the number of species with 1, and with 2, is nearly equal.

In the genus *Schizotricha*, the nematophores are similar in size and shape throughout but the number and position vary in the different species. This variation is due in part to the nature of the branching and the variation in the position of the nodes in the different species. For that reason, the internodal arrangement of the nematophores of the stem and branches differs in each species. In 2 species, *S. tenella* and *S. dichotoma*, where there is a hydrotheca in the axil of each branch and hydrocladium, the axillary nematophores appear as supracalcine nematophores, whereas, in the other two species, *S. parvula* and *S. gracillima*, where there is no hydrotheca in the axil, there is a single nematophore present instead. In each species there is 1 pair of supracalcine nematophores to each hydrotheca, but there is no agreement in the number of mesial nematophores between successive hydrothecae. In *S. tenella* and in *S. dichotoma*, there are 2 pairs of nematophores on the gonangium near the base, but in the other 2 species such have not been reported. There are no pseudonematophores.

In the genus *Polyplumularia* there is but 1 American species, *P. armata*, hence no specific comparison can be made. In the genus, the

lack of internodes on the stem and branches, and the large number of scattered, cauline nematophores present, makes the genus distinct from the others, and the unequal distances between the nodes in the hydrocladium, with the more than usual number of nematophores here as well, add to this distinction. The axillary and the supracalycine nematophores provide the only agreement. There are no pseudonematophores.

In the genus *Diplopteron*, the nematophores show something really distinctive in that, associated with each hydrotheca there are 2 pairs of nematophores, one pair distinctly lateral, and the other, supracalycine. The latter do not vary much in the 3 American species, but the former are of great enough difference in the different species, especially in length, to serve as good specific characters, those in *D. quadricorne* being almost twice as long as the hydrotheca.

Since nodes are absent in the stem and branches and may be absent in the hydrocladia, a comparison of the position of the nematophores, generally more numerous than in other genera, cannot readily be made.

D. longipinna carries the doubling up process to the extreme, since, instead of a single mesial nematophore proximal to the hydrotheca, there is a pair of them developed. In the only American species where the gonosome is known, *D. grande*, there is a pair of nematophores on the gonangium near the base.

In the genus *Hippurella* there is nothing very distinctive in the nematophores on the trophosomal part of the colony but in the protective structures for the gonangia, the modified, verticillate hydrocladia, without nodes or hydrothecae, but strongly nematophorous, the genus is unique. There are no nodes in the stem but, in *H. elegans*, there are scattered nematophores present, and in the axil of the hydrocladial process of each of the two American species, there is a nematophore distally placed and a pseudonematophore proximally. On the hydrocladium the pair of supracalycine nematophores and the single mesial nematophore on each node, thecate as well as athecate, show no innovations.

In the genus *Callicarpa* as well, the arrangement of the numerous nematophores in the phylactogonial branches is distinctive. In the 2 American species that have been described, the cauline nematophores are not mentioned and no figure of the stem has been given. The supracalycine nematophores are of the usual type in each species. In *C. gracilis* there is a single, proximal, mesial nematophore on the internode of the hydrocladium; in *C. chazaliei* this is also present but there is a distal, mesial nematophore as well.

In all of these eleutheropean genera of the *Plumularidae*, the dif-

ferences in the nature, position, and number of the nematophores are not sufficiently striking to make them of primary importance as generic characters. They are most useful, possibly, as specific characters, but even here their many differences in different parts of the same colony or in different colonies of the same species, may be as great as those in closely related species. As diagnostic characters in these genera, therefore, they can be safely used only as a confirmatory aid.

The one remaining eleutheropean genus, *Sphaerocystis*, of which there is but 1 species, *S. heteronema*, is in quite a different category from those already considered. It is provided with two types of movable nematophores, neither of which is similar to those appearing in other genera. The one type is bithalamic, with a slender proximal chamber which serves as a stalk for the larger, spherical, distal chamber. The second type is monothalamic, clavate, stout. Those of the bithalamic type appear on the stem, 1, a short distance distal to each hydrocladial process, and 1 in the axil of each process. On the hydrocladial internode there is a pair lateral to the hydrotheca, and a single mesial, directly above the hydrotheca. One large monothalamic nematophore appears on each hydrocladial internode, proximal to the hydrotheca, standing on a distinct shelf in the face of the internode. One smaller nematophore is situated on each hydrocladial process, distal to the bithalamic nematophore that appears in the axil.

To connect the eleutheropean and statopean genera there is the intergrading genus *Halopteris*, with the single species, *H. carinata*, in which some of the nematophores are movable and others are fixed.

The small, movable nematophores are shaped like those of the regular bithalamic type but they are monothalamic; there are 1 proximal and 1 distal to the hydrotheca in the hydrocladial internode, and 1 on the single, proximal, athecate internode. The lateral nematophores are unique. In place of the papilla that so often forms the support for a lateral nematophore, there is a more elongated, tubular process, from the upper surface of which, near the distal end, the nematophore grows out, at first as a tubular portion which gives rise to a cup-shaped terminal chamber, no part of it freely movable; the cup extends beyond the margin of the hydrotheca. In the axil of each hydrocladium there is a hydrotheca provided with similar lateral nematophores. On the cauline internode there are 2 small fixed nematophores and 3 similar ones on the other side. On the hydrocladial internode, in the angle between the hydrotheca and the internode, there is a small pseudo-nematophore.

The Statoplea. In the Statoplea there is a greater variety in the

nematophores, not so much in the number and the position as in the shape and size, not only in different genera and species but often in different parts of the same colony.

They appear in the simplest form in the genus *Tetranema*, with the single species, *T. furcata*, where they are all similar, shaped like the distal chamber of the nematophores of *Plumularia* but fixed, and with nothing corresponding to the proximal chamber. On the stem, they are present only on the hydrocladia-bearing tube, but there are several of them between 2 successive hydrocladia on the same side. On the hydrocladium, there are 2 pairs lateral to the hydrotheca and a single mesial nematophore some distance proximal to the base of each hydrotheca.

In the genus *Nuditheca*, with the single species, *N. dalli*, the trophosomal nematophores are shaped like those in *Tetranema*, although at least some of them have a partial internal septum, that almost divides the nematophore into 2 chambers. There are supracalcine nematophores adjacent to the hydrothecae in the axils of the branches, and 2 or 3 on each internode of that portion of the main branch of the hydrocladium that bears the branchlets. The cauline nematophores are numerous. On the hydrocladium, the single pair of nematophores are supracalcine, not lateral. The mesial nematophore is so close to the base of the hydrotheca that it passes for a short distance up the face. There is a pair of nematophores on the gonangium near the base. These are definitely bithalamic, looking like those in *Plumularia* but according to Nutting they are not freely movable.

In the genus *Diplocheilus*, with its single American species, *D. allmani*, the nematophores are like those in *Tetranema* except that they are much foreshortened. There is none on the cauline internodes except the single axillary nematophores. On the hydrocladial internodes there are no paired nematophores. There is a single mesial at the base of the hydrotheca and a small pseudonematophore between the hydrotheca and the internode.

In the genus *Halicornaria*, there are few differences in the nematophores in the different species. In the 3 species, *H. sinuosa*, *H. speciosa*, and *H. longicauda*, practically the only difference appears in the mesial hydrocladial nematophore at the base of the hydrotheca. In each of them there are no cauline nematophores except those associated with the hydrocladial processes, 2 on the face of each and 1 on the back, all tubular and rather small. On the hydrocladial internode there is the pair of short, tubular, supracalcine nematophores. In each species, the mesial nematophore is slender, tubular, more or less adnate to the

face of the hydrotheca. In *H. sinuosa* and *H. speciosa* it reaches little, if at all, beyond the margin of the hydrotheca, in *H. longicauda*, it reaches much beyond.

In *H. variabilis*, the nematophores on the hydrocladial process are quite different; the 2 on the face are bilobed, the distal 1 of the 2 being at least twice as great in each diameter as the proximal, but even this is quite large. Nutting does not mention any nematophore at the back of the process but probably there is one. The supracalcine nematophores are similar to those in the other species. The mesial nematophore is like that in *H. speciosa* except that it is quite short when the colony is young and grows larger as the colony gets older, but never larger than that in *H. speciosa*.

There is nothing very special about the nematophores in the genus *Aglaophenoides*, with its single species, *A. mammillata*. The cauline nematophores are small, cup-shaped, one on the internode at the base of the hydrocladial process, and one above this, near the distal node. On the hydrocladial internode, the supracalcine nematophores are tubular but definitely curved and slender; the mesial nematophore is adnate to the base of the hydrotheca only, the free portion directed mainly outward; it is slender, and near the base of the free portion there is a definite constriction or partial septum.

Although the genus *Streptocaulus* starts the development of special structures to protect the gonangia, its nematophores are more of the type found in the more primitive statoplean species; they are tubular or spur-like, and not adnate to the internode or to the hydrotheca. In the 2 American species, *S. pulcherrimus* and *S. gracilis*, with the stems slightly fascicled, the cauline nematophores are confined to the hydrocladia-bearing tube. In the former there is a nematophore at the base of the hydrocladial process and usually 3 between 2 processes in succession. In the latter they are more numerous. Each of them has a partial septum and the distal part is broader than the proximal. On the hydrocladial internodes in both species there are 2 supracalcine nematophores and a mesial, near the base of the hydrotheca, but free from it. This nematophore has 2 openings, a terminal and a lateral. Besides these, in *S. gracilis*, there are 2 mesial nematophores distal to the hydrotheca. On the modified appendages that support the gonangia there are no hydrothecae but there are nematophores, corresponding to the supracalcine pair, on each joint.

Since the species that have been included in the genus *Aglaophenopsis* and those included in the genus *Cladocarpus* have no constant difference in any character, there is no necessity of retaining the genus *Agla-*

ophenopsis. *Cladocarpus* may regularly include all of these species and they are considered here on that basis.

Although there is some variation among the species in nearly every character, the variation in the nematophores is not very great. In the 23 American species, all, with the possible exception of *C. sigma*, bear cauline nematophores. Quite often in the proximal portion of the stem, that does not bear hydrocladia, these are spur-like or denticulate, and appear in 1 or more definite rows. In the distal portion, they are not so numerous, some of them commonly related to the hydrocladial processes. They are nearly always tubular but there is a noticeable exception in *C. hirsutus*, where some of them are very large, butterfly-shaped and crenulated on the upper margin. In *C. cornutus* and *C. verrilli*, the margin is crenulated but the nematophores are tubular. On the hydrocladia, the supracalycines are always relatively small, tubular, although the length-breadth ratio varies. Most of them are slender but in *C. paradisea* they are bracket-shaped. They vary in length from those that do not reach the margin of the hydrotheca to those that overtop it to some extent. In *C. cornutus*, *C. grandis*, *C. hirsutus*, *C. speciosus*, and *C. verrilli*, the margin is crenulated, in all of the others, entire. All of the mesial nematophores are short, with the exception of those in *C. savignyana* (which possibly should not be placed with this genus), and in some of the species they are little more than spurs. In about one-third of the species, the nematophore is free from the hydrotheca; in the others the adnation varies from very slight to complete. In *C. hirsutus*, *C. speciosus*, and *C. verrilli*, the margin of the aperture is crenulate.

In the phylactogonia, the frequency of appearance varies much in the different species but the nematophores vary little in shape, as they are nearly all tubular or spur-like. In *C. pourtalesi*, though, they are stout and in *C. grandis* they are cup-shaped.

In the genus *Lytocarpus* the nematophores are prominent but there is not much variation in the American species. There are 2 cauline nematophores to each internode or the portion corresponding to one. In *L. furcatus* and *L. grandis*, they both are large, triangular, with the angles rounded; in *L. philippinus*, they may both be triangular, but they vary much from that to tubular; in *L. ramosus*, they are tri-lobate rather than triangular and are relatively small; in *L. clarkei*, one is triangular and the other is flask-shaped, and in *L. curtus*, both are nearly tubular and are small. In every species there is a perforated process or pseudonematophore on the upper side of the hydrocladial process.

With the exception of those in *L. furcatus*, the supracalycine nema-

tophores are tubular; in *L. curtus* they scarcely reach the margin of the hydrotheca but in all of the others they overtop it. In *L. furcatus* each nematophore is forked with the 2 branches of different length, the larger overtopping the margin of the hydrotheca. In *L. philippinus* and *L. clarkei* there are 2 definite apertures, one terminal, one lateral; in some of the other species the 2 apertures run together. The mesial nematophores are all long and adherent to the hydrotheca for most of their length. In *L. furcatus* and *L. curtus* they reach but little beyond the middle of the hydrotheca; in *L. clarkei* they are somewhat longer but do not reach the margin; and in the other 3 they pass beyond the margin. The apertures correspond to the apertures of the supracalcine nematophores in the same species. There is little difference except as to number in the nematophores on the phylactogonia; they are all tubular or spur-like.

The variation in nematophores in the 5 American species of the genus *Thecocarpus* is readily comparable with that in *Lytocarpus*. Of the cauline nematophores there is but one tubular and one in the axil of each hydrocladial process in *T. distans*; in *T. bispinosa* there is a large nematophore above each process, a small one below, and a pseudo-nematophore on the process; in the other species there are numerous small nematophores, tubular. On the hydrocladial internodes, the supracalcine nematophores are all small, although those in *T. benedicti* are broader than the others; only in *T. myriophyllum* are they long enough to overtop the margin of the hydrotheca. The mesial nematophores are relatively short; only in *T. myriophyllum* are they more than one-third of the length of the face of the hydrotheca; in *T. benedicti* and *T. bispinosus* there are 2 of them, one of them adnate to the hydrotheca and the other proximal to this; in *T. benedicti* the adnate nematophore is septate, and in *T. bispinosus*, the free nematophore is. On the phylactogonia the nematophores are numerous in all species, tubular or spur-like, but in *T. benedicti* they are cup-shaped as well.

In the large genus *Aglaophenia*, with 46 species in the area under discussion, the nature of the nematophores does not provide a very significant part of the means of separating the species, although it may readily confirm the diagnosis of the species determined by other characters.

Of the cauline nematophores there are most commonly 3 to an internode or the portion corresponding to an internode, where no nodes are present. In the majority of species these are placed in close relation to the hydrocladial process, e.g., 1 in the axil above, 1 below the process, and 1 on the process, but 1 of these may be removed some distance on the main part of the internode itself. Often the 3 are

tubular, either all of the same size or of different sizes, but in *A. diegensis*, *A. struthionides*, and *A. transitionis*, there are 1 triangular and 2 tubular, and in *A. robusta*, there are 2 triangular and 1 tubular. In about one-fourth of the species there are 2 nematophores rather than 3, and they may be either tubular or cup-like; in *A. constricta*, there is but 1 in the axil of the process. In at least 3 species, *A. bicornuta*, *A. raridentata*, and *A. flowersi*, there is a perforated process or pseudo-nematophore on the hydrocladial process.

Of the hydrocladial nematophores, there is not much variation in the supracalcine pair; each of them is tubular or nearly so, but it may be straight or curved, and may vary in the length-breadth ratio; in 6 species it is too short to reach the margin of the hydrotheca, in 18, just even with the margin or very nearly so and in the others overtopping the margin, the amount varying from very little to fully one-half of the length as in *A. curvidens*. Two apertures have been observed in at least 3 species, *A. cylindrata*, *A. allmani*, and *A. praecisa*.

The mesial nematophore shows more variation than any of the others and it may be quite significant in diagnosis. All of them are tubular, commonly tapering slightly to the distal end. They vary much in length from those in *A. minima* and *A. gracillima*, that are only about one-third of the length of the face of the hydrotheca to those in *A. allmani*, and still more in *A. ramulosa* that reach beyond the margin of the hydrotheca. They vary in degree of adnation, although most of them have a small portion free, and in the angle at which the free portion projects from the face of the hydrotheca. Those in *A. bicornuta* are unique. In place of the single mesial nematophore, there are 2 nematophores, 2 slender tubes placed side by side, like the barrels of a double-barrelled shot gun, and protruding downward as well as outward, the free ends being somewhat divergent. The free portion is about as long as the width of the hydrotheca.

In the gonosome, the nematophores form a regular series, on the margin of the corbula leaves, differing slightly in size and shape, spur-like, tubular, or deep cup-shaped, but they all give much the same appearance to the margin of the leaf.

In the Statopnea, therefore, although the nematophores are often much more conspicuous, when it comes down to the final analysis, they are of not much more importance in diagnosis of genera and of species than they are in the Eleutheropnea.

SUMMARY

In American hydroids, nematophores appear in all the genera and species of the one family, *Plumularidae*, and in certain genera of 3

other families; in the genera, *Lafoeina*, *Egmundella*, and *Oplorhiza*, in the *Campanulinidae*; in the genus *Ophiidissa*, in the *Halecidae*; and in the genera, *Eucryptolaria*, *Lictorella*, and *Zygophylax*, in the *Lafoeidae*. In these 3 families, except in the genus *Lafoeina*, the nematothecae are primitive, showing relatively little differentiation. Their development does not seem to be at all in unison with the evolution of other characters in these families. In some cases a nematophorous species may resemble a non-nematophorous species to a greater extent than it resembles any other species that has been placed in the same genus because it bears nematophores. Since this is the case, can a classification into genera in so far as it depends upon the presence or absence of this single character be considered a natural classification?

In the *Plumularidae* the case is somewhat different. Here the nematothecae vary in development from the very primitive type present in the perforated process or pseudonematophore to the complex statoplean type as it appears in both cauline and hydrocladial nematophores of such genera as *Thecocarpus* and *Aglaophenia*.

In a general way, there is justification for making a division of the family into the Eleutheroplea and Statoplea, even if there is an intergrading between the two, since many other characters vary correspondingly to give countenance to such a division. Going further, although the statement cannot be made all-inclusive, the nematothecae in the species of each genus that has been established are more nearly alike than those in species of different genera, and some of the nematothecae that show the greatest complexity are to be found in the most highly developed genera.

When individual cases are examined, it is evident that such a statement cannot be made concerning all of the nematophores. For instance, the very primitive nematotheca, in the perforated process, appears in species scattered through various genera, both eleutheroplean and statoplean, but often does not appear in closely related species.

Even in this family, then, there is plenty of evidence that the evolution of nematophores has not been synchronous with the evolution of other characters. It may be that some day the course of evolution in the nematophores will be worked out with some degree of definiteness, but, even if that is accomplished, there is no assurance that there will be any very intimate correspondence with the evolution of the families, genera, and species.

While the nature of the nematophores can be of much use in checking diagnosis of genera and species, it does not seem safe to use this as a single criterion for separating genera or species.

ON METHODS OF ESTIMATING THE SIZE OF PULMONIC
ALVEOLI AS DIAMETERS AND AREAS OF THEIR
OUTLINES IN 25μ SECTIONS OF LUNGS FIXED
AT A STANDARD DEGREE OF EXPANSION¹

By W. STANLEY HARTROFT and CHARLES C. MACKLIN, F.R.S.C.

THE use of sections to determine pulmonic alveolar size. Only those alveoli in limited subpleural regions of lungs of living animals of a few species have been directly observed, and then only by skilled workers using highly specialized techniques which have so far not been applied to man. Reconstructions by the Born (1) method take much time, and hence measurements therefrom are restricted in number and scope. Alveolar casts do not represent alveolar size accurately. These methods are thus not practicable in a survey of the lungs as a whole for alveolar size, and are not as suitable as is the section method for the solution of related pathological problems. Hence methods of estimating alveolar size were developed which are based on direct measurements of alveolar outlines as seen in microsections made from lungs fixed in a controlled state of expansion. They are relatively rapid and provide for the measurement of large numbers of alveoli from many different regions of the lungs. By averaging such data a good idea of the size of alveoli as defined in the title in any pair of lungs, such as that of man, may be gained in a reasonable time. Then, too, by applying the method to a series of lungs of different animals fixed in the same state of expansion, it is possible to make a comparison of the sizes of their alveoli: for any errors involved are constant throughout the series. Furthermore, from the data derived in this way, computations of alveolar capacity and wall surface may be made (2).

Two standardized methods of fixation were used, intrabronchial (3) and intravascular (4), which preserved all lungs, fixed by the same method at a practically uniform degree of expansion. The optimum section thickness for all lungs was found by trial to be 25μ .

The chief components of the optical fields of such sections are the split alveolar ducts and sacs, bordered by their rows of alveoli, and fields of alveoli cut tangentially, which show a foam-like structure. It is important to distinguish between the alveoli and the much larger sacs

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and ducts, of which they are but peripheral parts, for it is only the alveoli that are to be measured. Fig. 1 makes the relations clear.

The two types of alveolar outlines—"open" and "closed." An alveolus is a cup- or box-like outpouching from an alveolar sac or duct. An imaginary line, or central axis, leading from the midpoint of its base through that of its mouth would project toward the centre of the space of its alveolar sac or duct. As the alveolar axes lie at

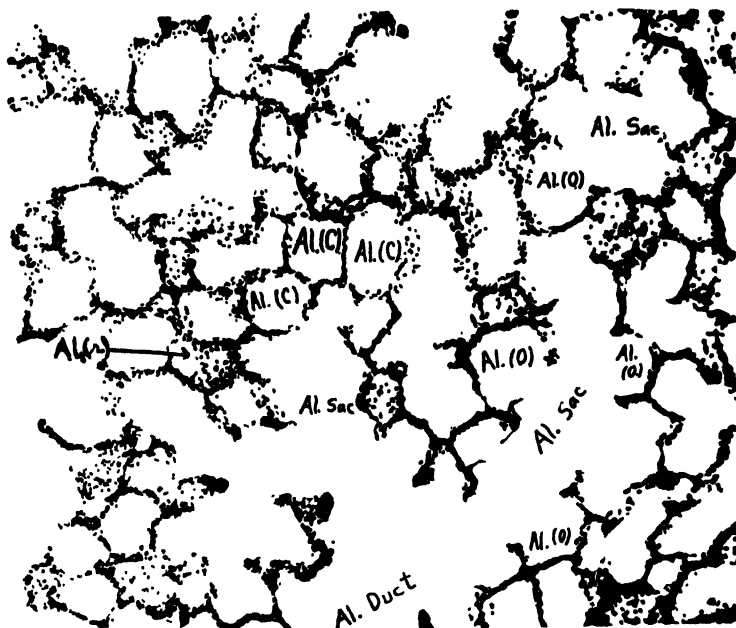


FIGURE 1.—Photomicrograph of section of lung of monkey (*M. Rhesus*) (S73-22M-IRU-no. 3) X 92. Abbreviations in the figure are defined as follows:

Al. (C)—Closed alveolar outline.

Al. (O)—Open alveolar outline.

Al. (r)—Alveolar outline with shelving edges which is rejected for measurement purposes.

Al. Duct—Alveolar duct.

Al. Sac—Alveolar sac.

every conceivable angle in relation to the stroke of the microtome knife, the alveoli are cut at an infinite variety of slants and so present a wide assortment of outlines in the microsections. These, however, can be set in two classes, open and closed. If the knife passes in any plane which includes the mouth, as, *e.g.*, in *b* of Fig. 2, the resulting outline

is open, or roughly U-shaped (b_1). If, however, the knife does not pass through the mouth, but skims the alveolar sac or duct tangentially, as in c of Fig. 2, the resulting outline is closed (c_1). The relative proportion of the open and closed types is influenced largely by the degree of expansion of the alveoli, the saucer-shaped and wide-mouthed alveoli of highly expanded regions of lung yielding, in sections, relatively more of the open outlines, whereas the glove-finger-shaped alveoli of collapsed areas, with their contracted mouths, yield relatively more outlines of the closed type.

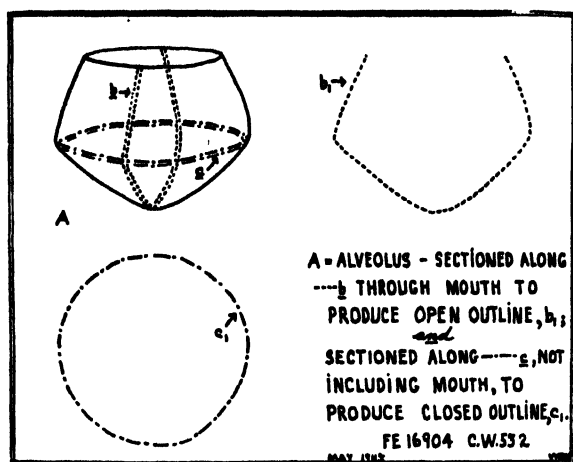


FIGURE 2.—Diagram to illustrate the manner in which an alveolus may be sectioned to produce open or closed outlines.

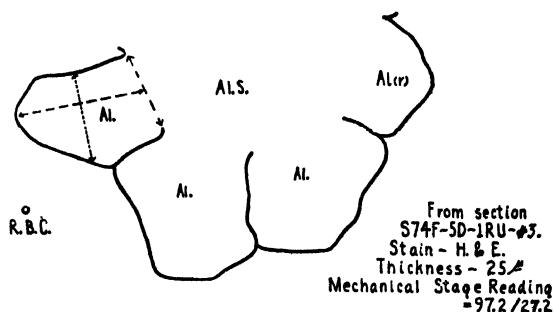
Selecting and tracing the alveolar outlines. Alveolar size may be determined from measurements of outlines of either the open or the closed type alone, or from a combination of these. Each type is measured by itself. To ensure the greatest accuracy, there must be adequate sampling of the lung tissue. First, what may be called gross sampling is done, a block being taken (3) from a standard area of each of the seven lobes or lobar regions (eight in guinea pig): and second, microscopic sampling is effected, 200 outlines of each type being selected from microsections from each lobar region. In this way representative data are gathered from all lobar regions of the pair of lungs of each animal studied. This method of widespread and numerous sampling tends to smooth out inequalities in alveolar size results depending on variations in degree of alveolar expansion in the different lobar regions.

These outlines are first traced on rolls of adding machine paper at a magnification of 320 diameters with the aid of a standard compound microscope provided with a camera lucida and a mechanical stage (Plate I). Starting at its lower left-hand corner, each section is systematically searched and every suitable outline (open or closed as the case may be) encountered is traced until the total of 200 of each type for each lobar region has been reached—1,400 per lung. The position of each tracing, as registered by both the horizontal and vertical scales of the mechanical stage, is recorded beside it, so that its original in the section may be found again at any time. Any outlines, open or closed, which possess thick sloping walls, lying at an acute angle to the surface of the slide, rather than at approximately ninety degrees, are the result of the sectioning of peripheral portions of the alveoli, and thus do not represent true alveolar size, being too small. Such outlines are,

ILLUSTRATING METHOD AM1, MEASURING OUTLINES OF ALVEOLI OPENING INTO ALVEOLAR SACS.

NRC M1001

FE 16904 C.W. 532.



The figure shows a camera lucida tracing at a magnification of 240 diameters, of outlines of four sectioned alveoli (AL) opening into an alveolar sac (ALS) taken from a dog lung preserved in the state of expansion by intrabronchial filling with fixative. The dimensions measured by method AM1 are depicted on the first alveolar outline on the left. They are represented and defined as follows:—

Mouth ---- Opening of alveolus into alveolar sac.

Depth ---- Greatest diameter from midpoint of mouth.

Width Diameter at right angles to depth at its midpoint.

The alveolar outline indicated by AL(r) is from an alveolus sectioned off center, appearing unduly small; all such are rejected for measurement.

For comparison, in the lower left corner, at the same magnification is shown a human red blood corpuscle with a diameter of 7.5 microns.

FIGURE 3.—Method AM1 of measuring outlines of alveoli opening into alveolar sacs. Reduced to approximately 160 diameters.

therefore, rejected (Al.(r) Fig. 1, 3). It is because they enable such rejections to be made easily and quickly that the thick 25μ sections are used.

Measuring the tracings. Primary estimations of size of the above mentioned tracings of the alveolar outlines may be made by measurement in two ways, (a) of selected dimensions, diameters or axes, and (b) of their areas. Whereas any one method will suffice to give data on alveolar size that will answer very well for comparative purposes, a combination of methods, assisted by calculation, leads to closer approximations of actual alveolar size in terms of dimensions or diameters (by combining linear data of open and closed outlines); or of area (by combining planimetric data from each type of outline); or of alveolar capacity or surface area (by calculation from data on diameters and areas).

The AM1 dimensional method: a comparative yardstick. The single method for direct comparisons of alveolar size which has been used throughout the series of laboratory animals and man is known as AM1 (Fig. 3). It uses open outlines and is reasonably rapid. Three standard dimensions, "mouth," "depth," and "width," are measured directly with a metric scale to the nearest millimeter, and are defined as follows:

Mouth (M)—the width of the opening by which the space within the alveolar outline is seen in the sections to communicate with that of its alveolar sac or duct (Fig. 1, 3).

Depth (D)—the distance from the midpoint of the mouth to the most remote point on the outline.

Width (W)—that diameter at right angles to the depth line drawn through its midpoint.

This method is primarily based on the mouth, the natural landmark of each open outline. The combined average of the means of depth and width (D-W Average) affords a standard comparative index or yardstick of pulmonic alveolar size in man and the common laboratory animals. It has proved useful in enabling us to arrange ten selected animal species in order of relative alveolar size thus providing an approach to certain physiological and pathological correlations. The dimension, mouth, is here used only as an essential step in orientating the other two dimensions, but might be used as an index of the degree of expansion of the alveolus when taken in conjunction with depth and width.

The AM1a dimensional method. This method (Fig. 4) uses open outlines as in AM1: but different dimensions called the maximum or

primary diameter (a), and the bisector or secondary diameter (b), are measured. a is the greatest diameter of the space enclosed by the outline when its mouth is closed by a straight line: and b is that diameter at right angles to a at its midpoint. Alveolar size is expressed as the average of the axes, or the " a - b average." In practice the values derived by this method are somewhat greater than those by AM1.

The AM4 dimensional method. In this method (Fig. 5), closed outlines are used. The maximum diameters and bisectors are known as X and Y respectively. X is the greatest diameter of the space enclosed by the outline, and Y the diameter at right angles to X at its midpoint. To express alveolar size, the average of the axes, spoken of as the "X-Y Average," is taken. The values derived by this method were in practice somewhat greater, as a rule, than those of AM1a.

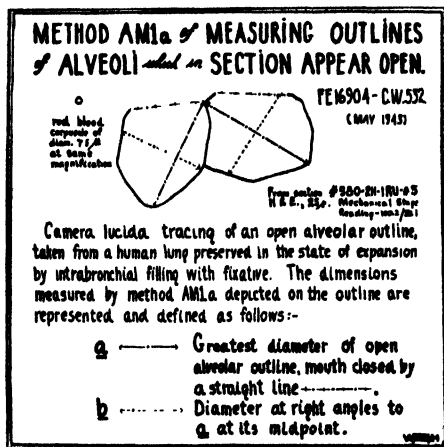


FIGURE 4.—Method AM1a of measuring outlines of alveoli which in section appear open. Two adjoining outlines of alveoli opening into a common alveolar sac are shown. Reduced to approximately 160 diameters.

Averages of AM1a and AM4. Since these methods are basically similar, their derived data may be averaged, and the result is spoken of as the "Combined a - b : X-Y average." It is felt that this combined average is a close approximation to actual alveolar size as expressed in terms of a linear dimension.

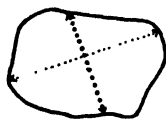
Measuring the outline areas. Alveolar size may be expressed in terms of the average of areas of either the open or the closed outlines. The method of making the enlarged tracings is as already described: indeed the same tracings may be used for areal measurement as for

dimensional. With a planimeter (that used by the authors was of Swiss manufacture—Plate II) each tracing is carefully followed completely. In the case of the open outlines the line representing the mouth is treated as part of the boundary, making the outline, for this practical purpose, closed. Each type of outline is measured by itself, and the results are kept separate. A magnifying lens is used to facilitate accurate readings to the nearest one-tenth of a square centimetre of the areas

ILLUSTRATING METHOD AM4, of MEASURING OUTLINES of ALVEOLI *which in* SECTION APPEAR CLOSED.

NRC M1001

FE 16904 CW.532.



Human red blood corpuscle •

From section
S74F-5D-1RU-3.
Stain - H. & E.
Thickness - 25 μ
Mechanical Stage Reading
- 98.7 / 26.7

The figure shows a camera lucida tracing, at a magnification of 240 diameters of an outline of an alveolus which appears closed; its opening into its alveolar sac not being included in the section, taken from a dog lung preserved in the state of expansion by intrabronchial filling with fixative. The dimensions measured by method AM4 depicted on the outline are represented and defined as follows:-

X • Greatest diameter of closed alveolar outline.

Y • Diameter at right angles to X at its midpoint.

For comparison, in the lower left corner, at the same magnification is shown a human red blood corpuscle with a diameter of 7.5 microns.

FIGURE 5.—Method AM4 of measuring outlines of alveoli which in section appear closed. Reduced to approximately 160 diameters.

enclosed by the outline tracings made at the standard magnification of 320 diameters. Averages of the areas of each type of outline are computed for each group of 200 observations per lobar region, and from these the mean for the entire lung, based on 1,400 tracings, is calculated. It is felt that actual alveolar size is more closely represented when a combined average of data from both open and closed outlines is taken than when the average of either of these types is considered by itself. These areal determinations may be used as a basis for calculating alveolar capacity (2). When made on the same outlines the results are confirma-

tory of those obtained by calculation from dimensional measurements.

Summations, averages, and statistical treatment of data. Each set of 200 measurements per lobar region (diametric or areal as the case may be) is totalled with the adding machine in two separate groups composed of the first 100 and of the second 100 measurements in order as taken. Each total is then divided by the number of measurements (100) and converted into microns, or square microns, and the value reduced to actual alveolar size as expressed in diametric or planimetric terms respectively. The average for the total 200 observations is similarly calculated. This method of dividing the data makes a system of complete checking of all calculations possible. From these lobar averages, the total lung mean based on the entire set of 1,400 observations can be computed and checked. All calculations are carried to two decimals. Frequency tables are constructed for each set of 1,400 measurements, showing the distribution of the observations according to graded class intervals of size. From these, the means for the entire lung are calculated a second time as a check on the first method. These tables are also utilized to compute the standard deviations² and probable errors³ of the means. Frequency polygons are plotted from the tables to give a graphic picture of the variation and distribution of the observations, the ordinates of each graph representing the number of observations, and the abscissae, the value of the observations in microns or square microns, as the case may be. The data could be subjected to still further statistical analysis, but that described above is considered sufficient for present purposes.

From the diameters, a , b and X , Y of the open and closed outlines respectively, their areas may be approximated by the familiar formula expressing the area of an ellipse in terms of its major and minor axes. That is, an estimation of the area of an open outline is given by

$$\pi \frac{ab}{4},$$

²"The standard deviation is the square root of the mean of the sum of the squares of all deviations, the deviations, of course, being measured from the arithmetic mean or average of the observations" (Woods and Russel, *An Introduction to Medical Statistics*, (London, P. S. King & Son, Ltd., Orchard House, Westminster, 1936, p. 67) ; or "Arithmetically the standard deviation is the square root of the arithmetic mean or average of the squared deviations of the observations from the mean of the distribution" (Raymond Pearl, *Introduction to Medical Biometry and Statistics*, W. B. Saunders Company, 1940, p. 354).

³ 0.67449 times the standard deviation divided by the square root of the number of observations: according to equation, p. 124, Woods and Russel (see above) : and according to formula, p. 350, Raymond Pearl (see above).

and of a closed outline by

$$\pi \frac{XY}{4}.$$

These alveolar outlines are not, of course, true ellipses, but both open and closed types may be regarded as approaching such.⁴

The average of areas computed in this way was found to agree, within reasonable limits, with the corresponding average of areas found by planimetric measurements.

Possible sources of error. As a uniform technic has been used for the material all along the line, it is felt that the results are reasonably reliable for comparative purposes—that is for gaining a conception of the relative size of pulmonic alveoli in a series of laboratory animals and man. Shrinkage inevitably occurs in the processing, but for comparative purposes need not be corrected, unless it is materially greater in some animals than others, and we have found no evidence of this. The error inherent in the random section method, due to some sections doubtless having been included which are less than the representative size (because, e.g., of their having been cut a little to one side of the central or widest part of the alveolus), probably occasions a somewhat lower figure for alveolar size than would be the case could we always select the sections showing the greatest area for any particular alveolus, and hence yielding the greatest average diameter. However it is reassuring to note that the method of selection of outlines as they are seen under the microscope has made possible the rejection of all those small, lateral slices which betray themselves from their “shelving” appearance; and we feel that, for practical comparative purposes, again, the data yielded are reliable.

For a final refinement of the figures of average alveolar size, computed on the basis of primary and secondary diameters, or of sectional area, or of volume, correction should be made for both shrinkage and the inclusion of undersized sections. The first, to be most effective, should be applied to each animal. Much can be done, for the second, by mathematical formula, and this will be considered in a later paper.

For a broad treatment of the possibilities of these methods in biological work reference may be made to the recent paper of Cole (5).

⁴The above application of this formula was made by one of us (W.S.H.) at the suggestion of Dr. R. H. Cole of the Mathematics staff of the University of Western Ontario.

SUMMARY

(a) The mode of use of specially prepared random 25μ sections from standard areas of all seven lobes (or corresponding regions) of mammalian lungs, preserved in a standard state of expansion, to determine pulmonic alveolar size, has been described.

(b) Some of the advantages of random section methods for the determination of pulmonic alveolar size have been pointed out.

(c) In sections, two types of alveolar outlines are encountered: "open" and "closed." The manner of selecting and tracing with a camera lucida those of each type which are suitable for alveolar measurement purposes is described.

(d) The single method, "AM1" adopted for direct comparisons of alveolar size, utilizes only open alveolar outlines. Three dimensions, "Mouth," "Depth," and "Width," are defined and measured for each outline. The Depth-Width average constitutes a comparative yardstick of alveolar size.

(e) Methods, termed AM1a and AM4, for estimating the sizes of open and closed outlines, respectively, have been developed, and comparisons of one with the other have been made. They are based on measurements of the greatest or primary diameters, and bisectors or secondary diameters; and these are termed a and b for the open outlines and X and Y for the closed. A combined average, $a-b:X-Y$, obtained from the averages of the open ($a-b$) and closed ($X-Y$) outlines gives a close approximation to true alveolar size expressed in linear terms.

(f) A method for measuring the areas of both types of outline with a planimeter is described.

(g) All methods call for measurements of 1,400 outlines for each pair of lungs. The manner of summing and averaging the data is given. Statistical treatment necessary for estimating the significance of the results is indicated.

(h) A method for calculating the areas of alveolar outlines (open or closed) from their primary and secondary diameters has been worked out, and formulae for this purpose are given. Results obtained are reasonably similar to those derived from direct planimetric measurements.

(i) Possible errors in these methods due to shrinkage of the lung tissue and inclusion of measurements of undersized sections of alveoli are considered.

(j) An adequate sampling of lung tissue is obtained by these methods. It is the merit of the random microsection method that samples may be taken from all parts of a pair of lungs and measured

for alveolar size, thus ensuring a much more thorough survey of the tissue than is possible by other methods.

(*k*) Alveolar size may be conceived of in terms of one, two or three dimensions. Unidimensional size may be expressed in terms of the average of two dimensions chosen in different ways as (*a*) orientated to the mouth—AM1: (*b*) primary and secondary diameters of open, AM1a, or closed, AM4, outlines of alveoli, or combined average of AM1a and AM4. Bidimensional size may be taken as the average of the areas of random slices through open, or closed, outlines, or may be the combined average of both of these. Tridimensional size, by the random section method, is arrived at through mathematical treatment.

(*l*) The application of these methods to lungs of man and laboratory animals has yielded information of value from the physiological, pathological and comparative anatomical standpoints. The "base-line" afforded by normal alveolar size determinations in man would be useful, for instance, in estimating the extent of departure from normal in emphysema.

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The authors wish to thank Mr. Charles Jarvis for the photographic work.

EXPLANATION OF PLATES

PLATE I

Microscope with camera lucida attachment and rolls of adding machine paper used to trace outlines of alveoli for purposes of measurement.

PLATE II

Illustrating the method of measuring the areas of camera lucida tracings of alveolar outlines, by means of a planimeter and magnifying lens.

PLATE I

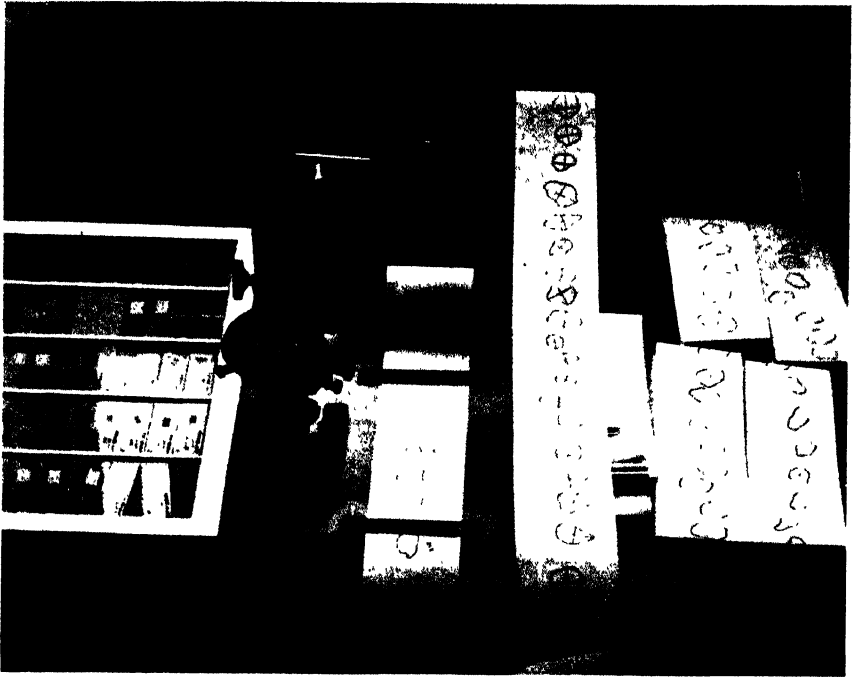
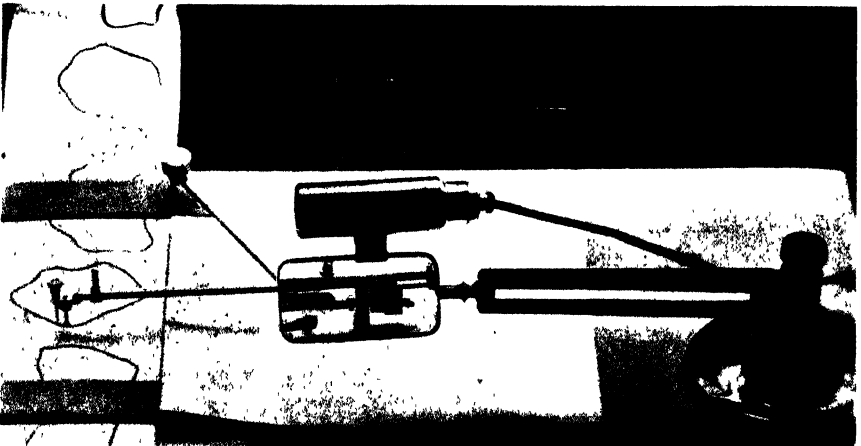


PLATE II



THE SIZE OF HUMAN LUNG ALVEOLI EXPRESSED AS DIAMETERS OF SELECTED ALVEOLAR OUTLINES AS SEEN IN SPECIALLY PREPARED 25μ MICROSECTIONS¹

By W. STANLEY HARTROFT and CHARLES C. MACKLIN, F.R.S.C.

THE lungs (S80-2H) were from a woman aged thirty-one. Congestion and incomplete collapse in the posterior and inferior portions were the only significant changes noted at autopsy. All procedures of fixation and mensuration are as previously described (1, 2). Control dimensions of the interior of the pleural cavity were 23.1 cm. on the right side and 23.8 cm. on the left. The exact hour of death is unknown. Fixation was done as soon as possible, but the delay was probably about 12 hours. The intact heart and lungs were placed in a bath of 3 per cent formalin and 3,500 c.c. of Bouin's picric-formol solution was gradually introduced *via* a tracheal cannula, as much air as possible being allowed to escape. From apex to paravertebral part of inferior border the lungs then measured 26.7 cm. for the right and 27.2 cm. for the left. These lengths are approximately 3.5 cm. in excess of the corresponding pleural cavity dimensions. This overfilling was deliberately done to compensate for expected shrinkage due to loss of fluid through the pleura and from the slices after cutting. The lungs were allowed to remain in the formalin bath for eleven weeks. The measurements (taken 42 times) remained practically unchanged from date of bronchial filling to that of slicing. Each lung was then cut in a special mitre box with a brain knife into transverse slices 3 cm. in thickness and blocks were selected from seven standard regions representing the seven lobes of mammalian lungs including the cardiac (1) or intermediate lobe. Sections were cut at 25μ and stained with hematoxylin and eosin.

Two thousand, eight hundred alveolar outlines were traced with the camera lucida, 400 from sections of each of the seven regions, one-half the number being closed and one-half open in type (2). The open outline dimensions were measured by two methods, AM1 and AM1a,

¹This work was done under grants from the National Research Council of Canada and the Department of National Defence, Canada. Presented as part of paper 43 before Section V of the Royal Society of Canada at McMaster University, Hamilton, Ontario, on May 26, 1943. See Abstract on p. 137, Proc. Roy. Soc. Can., 1943, ser. 3, 37. Dr. Hartroft, in his capacity as Research Assistant, has been responsible for the preparation of the material and the execution of the mensurational, mathematical, and statistical work.

and the closed by method AM4 alone. Descriptions of these methods and the statistical treatment of results so obtained have already been presented (2).

The histology of the expanded human lung, as seen in sections, is so much like that of the monkey that Fig. 1 of the preceding paper (2) will suffice to make clear the meaning of the various structural terms used.

It is important to recall that the alveoli are but small bays or sacculi opening by their "mouths" into larger cavities known as alveolar sacs and ducts. Alveoli are fitted closely together in a manner suggesting the cells of a honeycomb. Their form is altered with inflation and deflation of the lung, the mouth diameter becoming greater in the distended condition and the depth relatively less. No attempt has been made to determine the extent of the space of the alveolar sacs and ducts in this study.

Method AM1. A diameter known as "Depth-Width," or "D-W," is yielded by this method of alveolar mensuration, which treats the mouth as a dimension, and orientates the depth and width lines with it. The depth, D, is the length of a straight line representing the distance from the midpoint of the mouth to the most remote point on the alveolar wall outline; and the width, W, is the length of a straight line drawn through the midpoint of D and at right angles to it from side to side of the alveolar outline. The average of these two lines, D-W, is a useful expression of alveolar size.

The D-W grand average for the measurements of 1,400 open alveolar outlines of all seven lobar regions of this adult human lung was found to be *166.11 microns*. Table I shows this final value and also, at the bottom, the D-W average values for the individual lobes from which the final grand average was derived.

The highest depth-width average is that for the right upper lobe and the lowest that for the cardiac lobar region. The block of lung from the latter part as well as those from the left middle, left lower, and right lower regions, were all selected from terminally congested areas, and the D-W averages for these are all lower than similar values of the right upper, right middle, and left upper regions, which were comparatively free from such congestion. These variations are all less than 24 microns, and are felt to be within normal limits.

Figures are given for the separate depth and width values, in each lobar region and for the entire lung, and the standard deviation and probable error are given for the total lung means of these dimensions.

The value averages for depth and width are shown in groups of

200, for each lobe, and for the entire lung (column on the right): and each of these groups has been divided, for checking purposes (2) into subgroups of 100.

The values for the mouth dimension are similarly presented in this table. The mouth grand average for this human lung was found to be

TABLE I
HUMAN S80-2H AM1 DIMENSIONS

All figures denote microns

Dimension	Group	Right upper	Right middle	Right lower	Cardiac	Left upper	Left middle	Left lower	Average
MOUTH	1st 100	135.44	133.41	127.94	118.69	127.06	117.25	118.56	125.48
	2nd 100	144.53	132.16	134.78	130.78	146.66	111.66	117.50	131.15
	Total 200	139.98	132.78	131.36	124.73	136.86	114.45	118.03	128.31
DEPTH	1st 100	188.66	190.03	177.84	166.41	184.44	190.50	175.44	181.90
	2nd 100	189.19	178.06	183.13	163.41	195.16	174.22	176.09	179.89
	Total 200	188.92	184.05	180.48	164.91	189.80	182.36	175.77	180.90
WIDTH	1st 100	158.44	166.56	151.41	136.34	150.06	139.88	141.41	149.16
	2nd 100	172.19	155.72	157.88	146.97	168.81	131.34	141.59	153.50
	Total 200	165.31	161.14	154.64	141.66	159.44	135.61	141.50	151.33
DEPTH and WIDTH Av.	Total 200	177.11	172.59	167.56	153.28	174.62	158.98	158.63	166.11

Standard deviation and probable error of mouth mean (total lung): 35.037 and 0.63160 microns.

Standard deviation and probable error of depth mean (total lung): 36.314 and 0.65464 microns.

Standard deviation and probable error of width mean (total lung): 35.410 and 0.63832 microns.

128.31 μ . Uses for this dimension, in association with the others, may be found, for instance, in pathology (3).

Table II shows the frequency distribution of the measurements averaged in Table I, the values for mouth, depth, and width being

TABLE II
AM1 METHOD OF MENSURATION
HUMAN S80-2H

Class units in microns	NUMBER OF OBSERVATIONS		
	M (Mouth)	D (Depth)	W (Width)
14.1 - 26.5			
26.6 - 39.0			
39.1 - 51.5	3		
51.6 - 64.0	14		
64.1 - 76.5	44		4
76.6 - 89.0	92	3	14
89.1 - 101.5	182	5	61
101.6 - 114.0	208	13	115
114.1 - 126.5	184	47	159
126.6 - 139.0	198	97	198
139.1 - 151.5	138	135	211
151.6 - 164.0	106	206	186
164.1 - 176.5	99	174	156
176.6 - 189.0	55	178	100
189.1 - 201.5	36	144	76
201.6 - 214.0	19	133	39
214.1 - 226.5	12	117	31
226.6 - 239.0	4	69	26
239.1 - 251.5	3	32	11
251.6 - 264.0	3	19	7
264.1 - 276.5		14	4
276.6 - 289.0		10	1
289.1 - 301.5		3	1
301.6 - 314.0		0	
314.1 - 326.5		1	
326.6 - 339.0			
339.1 - 351.5			

set down in vertical columns opposite their respective brackets in the column "class units in microns." Frequency curves, or "polygons," were plotted from these values (Fig. 1), and present their variation and distribution graphically. The ordinates stand for the number of observations and the abscissae for the value in microns. The dotted line represents the mouth dimension, the solid line, the depth, and the dash line, the width.

The D-W grand average by method AM1 has been found to constitute a valuable yardstick in comparing alveolar size in a series of animals. The value for man, 166.11 microns, was the highest in a series of mammalian lungs prepared and measured by AM1. Following man we have, in diminishing order of magnitude, the cat, rabbit,

monkey, guinea pig, goat, dog, baboon, rat, and mouse. It is not possible at present, to explain the meaning of these alveolar size variations in the mammals.

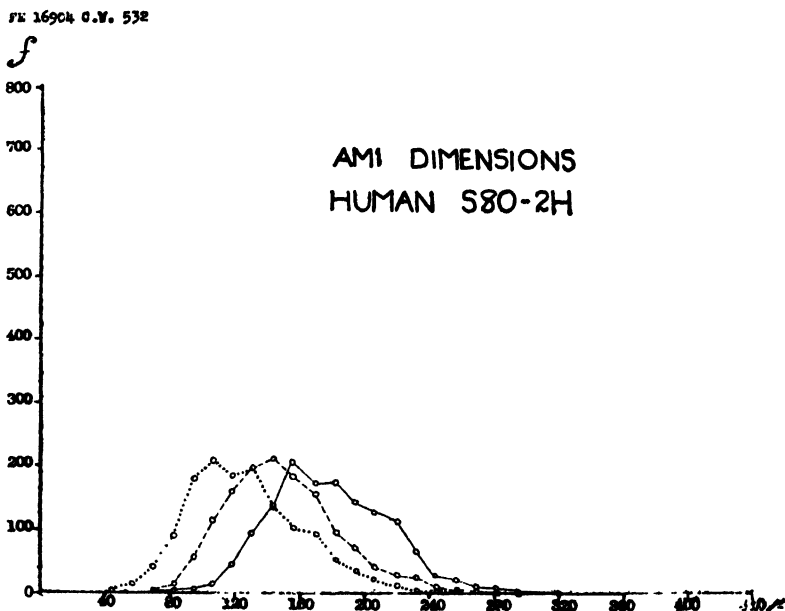


FIGURE 1.—Frequency polygon, constructed from data of Table II. Dotted line—"mouth"; Dash line—"width"; Solid line—"depth." Ordinates represent the number of observations: Abscissae, the values of the dimensions in microns.

Method AM1a. Using the same open outline tracings as those of AM1, but regarding the mouth dimension as part of the outline, the distance across the outline at its widest part is measured on a line called *a*. This is the maximum or primary diameter, or axis. Through the midpoint of *a* and at right angles to it, diameter *b*, also known as the bisector or secondary diameter, is drawn from side to side of the outline and measured.

Table III is made up of *a-b* dimension averages arranged according to lobar regions. As in Table I, they are shown in groups of 200 for each lobe, and each of these groups is further divided into two sub-groups of 100 each, for checking purposes. Method AM1a confirms Method AM1 in that the lobar regions with the three highest values are again right upper, right middle, and left upper. All lobar values are greater than the corresponding AM1 averages.

TABLE III
HUMAN S80-2H AM1a DIMENSIONS

All figures denote microns

Dimension	Group	Right upper	Right middle	Right lower	Cardiac	Left upper	Left middle	Left lower	Average
<i>a</i>	1st 100	229.16	236.72	217.06	208.41	228.59	232.31	216.81	224.15
	2nd 100	236.09	223.78	229.09	213.09	243.16	217.56	214.59	225.34
	Total 200	232.63	230.25	223.08	210.75	235.88	224.94	215.70	224.75
<i>b</i>	1st 100	158.47	160.78	147.22	134.72	147.44	142.81	136.88	146.90
	2nd 100	163.94	149.98	151.46	141.91	164.88	129.78	141.88	149.11
	Total 200	161.20	155.38	149.33	138.31	156.16	136.30	139.38	148.01
<i>a-b</i> AVERAGE	Total 200	196.91	192.81	186.20	174.53	196.02	180.62	177.54	186.38

Standard deviation and probable error for dimension *a* (total lung): 39.998 and 0.72103 microns.

Standard deviation and probable error for dimension *b* (total lung): 32.510 and 0.58603 microns.

The *a-b* grand average of the 1,400 open outlines from this lung is shown to be 186.38 microns. This value represents the alveolar size of this human lung by the AM1a method of mensuration.

Table IV shows the frequency distribution of the values appearing in Table III.

The frequency distribution has been graphically summarized in the polygon, Fig. 2. The *a* diameter is represented by the solid line, and the bisector *b* is seen as a succession of dashes.

Since the dimension *a* is the greatest diameter of the outline it is frequently longer than the greatest AM1 dimension, which in this human lung is most often D. The values of *b* are of the same order as those of W of AM1. It is thus apparent that the average of *a* and *b* will exceed the D-W average for any representative group of open outlines.

The AM1a method has been especially useful in combination with AM4, now to be described.

TABLE IV
AM1a METHOD OF MENSURATION
HUMAN S80-2H

Class units in microns	NUMBER OF OBSERVATIONS	
	<i>a</i>	<i>b</i>
14.1 - 26.5		
26.6 - 39.0		
39.1 - 51.5		
51.6 - 64.0		1
64.1 - 76.5		3
76.6 - 89.0		18
89.1 - 101.5		59
101.6 - 114.0		107
114.1 - 126.5	1	175
126.6 - 139.0	5	218
139.1 - 151.5	18	237
151.6 - 164.0	41	198
164.1 - 176.5	78	147
176.6 - 189.0	143	88
189.1 - 201.5	148	59
201.6 - 214.0	137	38
214.1 - 226.5	173	25
226.6 - 239.0	188	12
239.1 - 251.5	141	10
251.6 - 264.0	97	2
264.1 - 276.5	84	1
276.6 - 289.0	54	2
289.1 - 301.5	37	
301.6 - 314.0	28	
314.1 - 326.5	16	
326.6 - 339.0	3	
339.1 - 351.5	3	
351.6 - 364.0	3	
364.1 - 376.5	1	
376.6 - 389.0	1	

Method AM4. We come now to the measurement of the closed type of alveolar outline (2, Fig. 1). It has been explained that the same alveolus can be sectioned to show an open or closed outline, the result depending on whether the microtome knife passes through its mouth or not (2, Fig. 2). It hardly needs to be said that there are not two types of alveoli. On the tracings of the closed outlines made at the same magnification ($\times 320$) as that used for the open outlines, the greatest diameter X, also known as the maximum or primary diameter or axis, is drawn and measured. Through the midpoint of X and at right angles to it is drawn the bisector Y, a secondary diameter or axis.

The average of these dimensions—the X-Y average, has proved to be a useful index of alveolar size derived from microsections.

TABLE V
HUMAN S80-2H AM4 DIMENSIONS

All figures denote microns

Dimension	Group	Right upper	Right middle	Right lower	Cardiac	Left upper	Left middle	Left lower	Average
X	1st								
	100	237.81	237.56	210.91	215.41	229.88	247.78	231.06	230.06
	2nd								
	100	239.56	233.03	212.47	215.16	241.00	251.44	235.25	232.56
	Total								
	200	238.69	235.30	211.69	215.28	235.44	249.61	233.16	231.31
Y	1st								
	100	166.56	165.88	149.75	151.59	158.94	151.28	152.03	156.58
	2nd								
	100	170.34	162.41	147.69	147.78	166.31	149.00	150.63	156.31
	Total								
	200	168.45	164.14	148.72	149.69	162.63	150.14	151.33	156.44
X-Y AVERAGE	Total								
	200	203.57	199.72	180.20	182.48	199.03	199.88	192.24	193.88

Standard deviation and probable error for dimension X (total lung): 41.437 and 0.74697 microns.

Standard deviation and probable error for dimension Y (total lung): 31.083 and 0.56031 microns.

Table V shows the averages of the X and Y dimensions in groups of the first and second hundred measurements, and total two hundred measurements, for each lobe; and also the final averages for each group and subgroup, for each lobe, and for the entire lung. The standard deviation and probable error are given for each dimension, on the basis of the total lung mean.

The grand X-Y average for the 1,400 closed outlines from all seven lobar regions of this lung is *193.88 microns*. The values for the lower and cardiac lobes are the smallest, thus confirming the *relative* values of the sets of observations obtained by the AM1 and AM1a methods. It will be seen, on comparison with Table III, that the X-Y dimensions generally exceed those derived by AM1a (which also is a "greatest diameter" method of mensuration); and from this we may conclude that the greatest alveolar dimensions are those which do not

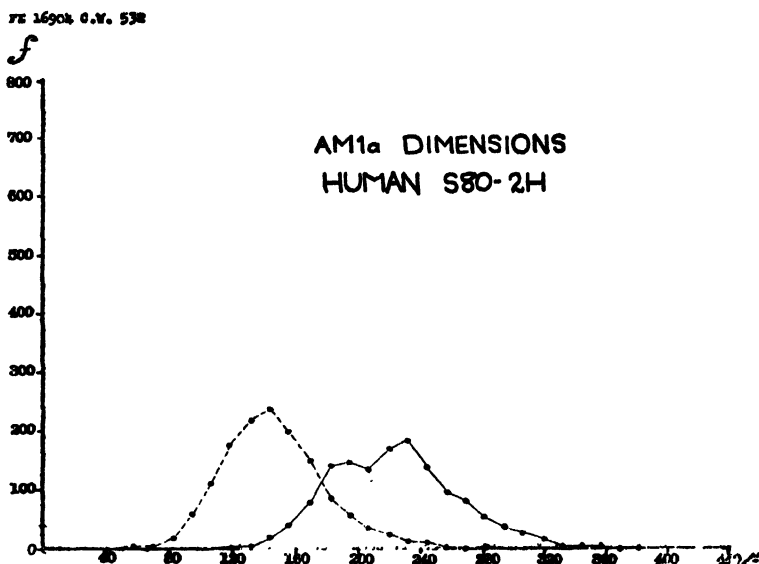


FIGURE 2.—Frequency polygon, constructed from Table IV. Dash line—*b* diameter; Solid line—*a* diameter. Ordinates represent the number of observations; Abscissae, the values of the dimensions in microns.

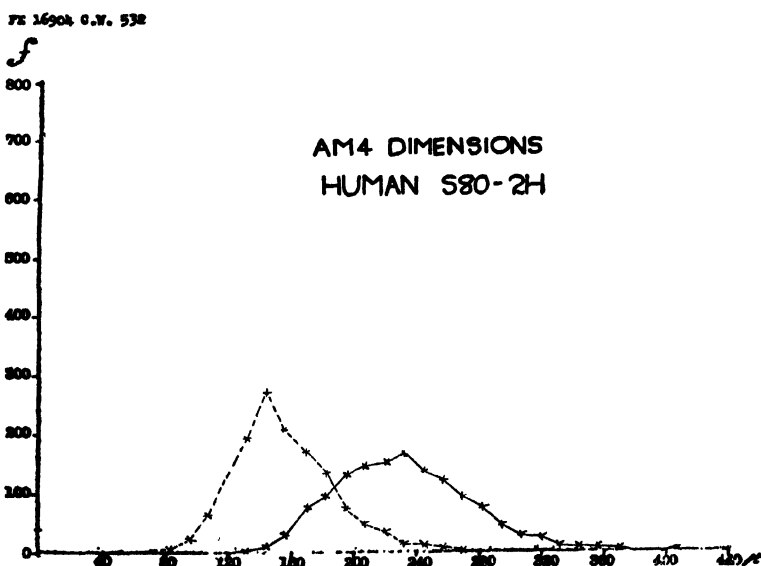


FIGURE 3.—Frequency polygon, constructed from data of Table VI. Dash line—*Y* diameter; Solid line—*X* diameter. Ordinates represent the number of observations; Abscissae, the values of the dimensions in microns.

TABLE VI
AM4 METHOD OF MENSURATION
HUMAN S80-2H

Class units in microns	NUMBER OF OBSERVATIONS	
	X	Y
14.1 - 26.5		5
26.6 - 39.0		22
39.1 - 51.5		64
51.6 - 64.0		129
64.1 - 76.5		195
76.6 - 89.0		272
89.1 - 101.5		208
101.6 - 114.0		172
114.1 - 126.5		137
126.6 - 139.0	3	77
139.1 - 151.5	9	47
151.6 - 164.0	32	38
164.1 - 176.5	79	14
176.6 - 189.0	99	12
189.1 - 201.5	135	7
201.6 - 214.0	152	1
214.1 - 226.5	159	
226.6 - 239.0	170	
239.1 - 251.5	140	
251.6 - 264.0	128	
264.1 - 276.5	97	
276.6 - 289.0	78	
289.1 - 301.5	48	
301.6 - 314.0	26	
314.1 - 326.5	21	
326.6 - 339.0	8	
339.1 - 351.5	6	
351.6 - 364.0	5	
364.1 - 376.5	4	
376.6 - 389.0	0	
389.1 - 401.5	0	
401.6 - 414.0	1	

pass through the mouth; or, in other words, the greatest alveolar dimension is included more often in closed than in open outlines.

The data from Table V have been subjected to statistical treatment, and the result appears in Table VI showing the frequency distribution. The greatest number of observations fall consistently into the modal brackets 189 to 264 microns for X, and 114 to 189 microns for Y. A graphic representation of this distribution is seen in the polygon of

Fig. 3, where the observations for X are plotted on the solid line, and those for Y on the dash line.

Combined AM1a and AM4 average. When alveolar size is to be expressed by a single linear dimension, that is most adequately done when that single value is based on measurements from outlines representing all planes rather than from measurements of planes passing only through the alveolar mouth (open outlines alone—D-W, or *a-b*), or only through alveolar parts other than the mouth (closed outlines alone—X-Y). Thus it seemed best to merge the data from both open and closed outline measurements, and this can be done justifiably by combining *a-b* and X-Y, since these diameters are obtained by the use of comparable methods AM1a and AM4. A simple average calculated from both the AM1a and AM4 final grand average figures possesses the advantage, then, of being based on measurements by comparable methods, representing the widest assortment of alveolar planes; and there is the additional advantage that the final average is based on twice as many observations,—2,800 rather than 1,400. Such a combined average is known as *a-b:XY*, and this was found to be *190.13 microns* for this human lung. It is regarded as constituting a universally representative average alveolar diameter, and the closest approximation to alveolar size expressed as a single linear dimension. Its attainment is, however, more time-consuming than is that for D-W, and the latter has been found quite satisfactory for the comparative use to which it has been put.

Possible sources of error. This subject has been discussed in the technical paper (2). The 15 per cent overfilling of the lungs at fixation was intended to compensate for later shrinkage of the tissue and diminution in alveolar size, but there was no attempt to duplicate exactly any given phase of the respiratory cycle—a difficult if not impossible feat. All that we have done is set a standard of procedure which can be duplicated in other lungs of man and in those of lower mammals, so that comparisons may be made.

It has been pointed out that alveoli from parts of the lung in a state of congestion are of smaller size than those in areas free from that change. The greatest difference, by the AM1 method, was only 24 microns—little more than the sum of the diameters of three human red blood corpuscles. It seems reasonable to assume that thickening of the alveolar walls due to swelling of their capillaries is an important factor in bringing about this small reduction in the linear dimensions of these alveoli.

Discussion. Alveolar size is, of course, a variable quantity, so that a statement as to the “normal” or “basal” size necessarily involves precise

definitions of many related conditions. Much more than in measurements of histological structural details of tissues such as bone, brain, or liver, pulmonic alveolar size data possess scientific meaning only when the many contributory factors, such as fixation, section technique, mensuration method, etc., are all clearly stated. Species and age are of great importance. It is felt, however, that the linear indices of human pulmonic alveolar size given in this paper are reliable for the age and conditions stated, and that they probably afford a close approximation to similar indices in the living body of like age. Any attempt, however, to correlate them finely with the precise size of living human alveoli at any given phase of the respiratory cycle would invite difficulties. These indices are, moreover, valuable for purposes of comparison with other human lungs similarly prepared and measured and with those of animals. They afford a beginning in the study of the variation in size of alveoli with age, in man. No attempt has been made here to review reports on other determinations of alveolar size in the literature, for the methods used in earlier studies differ from those used by us and also differ among themselves, so that comparisons would be unjustifiable; and not infrequently the methods are not stated. Earlier writers, too, have not been in agreement as to what an alveolus really is.

These expressions of human pulmonic alveolar size as single diameters may be applied to the solution of related problems in human and comparative histology, physiology, and pathology. Their importance in investigations of the degree of change in vesicular emphysema will be realized by the pathologist (3). In conjunction with determinations of total alveolar numbers in lungs, they may be used in calculations of total alveolar surface and total alveolar capacity.

SUMMARY

(1) The size of human lung alveoli has been found and expressed as diameters of selected alveolar outlines as seen in specially prepared 25μ microsections.

(2) These outlines have been separated into two fundamentally distinct types, open and closed. In the former the alveolus has been cut in a plane through the mouth opening into its sac or duct, while in the latter the alveolus has been cut in a plane other than that which includes the mouth.

(3) The diameter represented by the D-W grand average, obtained by method AM1, is *166.11 microns*. For simple comparisons utilizing only one type of outline (open) this index of alveolar size is satisfactory.

It has advantages of being easy to determine, and being correlated with the mouth dimension.

(4) The diameter represented by the *a-b* grand average obtained by method AM1a, is *186.38 microns*. This method also uses open outlines but its primary diameter is the greatest dimension, and thus it gives values somewhat higher than those of AM1.

(5) The diameter represented by the X-Y grand average by method AM4 is *193.88 microns*. Its values are somewhat higher than those of AM1a. Its results may be combined with those of AM1a as its axes are of the same type.

(6) The diameter represented by the combined grand average of *a-b* and X-Y is *190.13 microns*. This combined average has the advantages of being based on measurements theoretically in all possible planes of alveolar space, and of including twice as many measurements as AM1a or AM4 alone. We regard it as the closest approximation to human alveolar size in this specimen, expressed as a single linear dimension. It is thus an excellent unidimensional index of alveolar size, but requires much more time to determine it than do diameters by methods using one type of outline only. It may be utilized as an important factor in the calculation of total alveolar surface and total alveolar capacity.

(7) All figures given here for alveolar size in man are based on material fixed by bronchial filling (1). They are probably somewhat higher than they would have been had the fixation been by intravascular perfusion.

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THE SIZE OF HUMAN LUNG ALVEOLI EXPRESSED AS
AREAS OF OPEN AND CLOSED ALVEOLAR OUTLINES
AS SEEN IN 25μ MICROSECTIONS¹

By W. STANLEY HARTROFT and CHARLES C. MACKLIN, F.R.S.C.

IN the preceding paper (1) the size of human lung alveoli in a woman aged thirty-one was expressed as diameters of alveolar outlines as they appeared in specially prepared 25μ random sections (2). It is now proposed to represent the size of the same alveoli as areas of planes passing through them. These planes are established by the stroke of the microtome knife in traversing the paraffin block. They may or may not include the alveolar mouth. If they do, then the corresponding alveolar outline in the section is "open"; and if they do not it is "closed" (3).

The areas of 1,400 outlines of each type have been measured and averaged separately, and the averages have been combined with one another. The measurements were made with a planimeter (3), on the same camera lucida tracings ($\times 320$) used for the diametric determinations. As well as being thus directly determined by mensuration the areas of these same alveolar planes have been estimated by calculation from the combined average diameter (1).

Planimetric determinations from open outlines. The open outlines for the AM1 and AM1a diametric determinations of alveolar size in this human lung (S80-2H) were followed with the planimeter stylus, using the mouth line as a boundary, and the results have been set out in Table I in the form of itemized and grand averages. In each lobe 200 outlines were so measured and the table shows the averages of these lobar groups and also of their two component subgroups of 100 each. The column on the right shows the grand averages of these groups. The standard deviation and probable error of the mean open outline area for the total lung are given.

The grand average alveolar area for the entire lung, as measured on these 1,400 open alveolar outlines from all seven lobar regions, is

¹This work was done under grants from the National Research Council of Canada and the Department of National Defence, Canada. Presented as paper 44 before Section V of the Royal Society of Canada at McMaster University, Hamilton, Ontario, on May 26, 1943. See Abstract, p. 137, Proc. Roy. Soc. Can., 1943, ser. 3, 37. Dr. Hartroft, in his capacity as Research Assistant, has been responsible for the preparation of the material and the execution of the mensurational, mathematical, and statistical work.

TABLE I
HUMAN S80-2H AM1 PLANIMETRIC

All figures denote square microns

	Group	Right upper	Right middle	Right lower	Cardiac	Left upper	Left middle	Left lower	Average
AREA OF OPEN ALVEOLAR OUTLINES	1st 100	26591	28381	24273	20758	25646	24778	22524	24707
	2nd 100	29344	25092	25923	22284	29545	21236	22412	25119
	Total 200	27967	26736	25098	21521	27595	23007	22468	24913

Standard deviation and probable error of mean open outline area (total lung): 8630.8 and 155.58 square microns.

24,913 square microns, or almost 0.025 square millimetres. By comparing with Tables I and III of the preceding paper (1) it will be seen that the areal lobar averages confirm the linear averages for the same lobes, in that higher values are obtained for those blocks selected from the upper portions of the lungs.

TABLE II
AREA OF OPEN OUTLINES
HUMAN S80-2H

Class units in square microns	Number of observations
928 - 4833	2
4834 - 8739	36
8740 - 12645	165
12646 - 16551	269
16552 - 20457	283
20458 - 24363	229
24364 - 28269	171
28270 - 32175	95
32176 - 36081	67
36082 - 39987	34
39988 - 43893	21
43894 - 47799	16
47800 - 51705	6
51706 - 55611	3
55612 - 59517	3
59518 - 63423	
63424 - 67329	
67330 - 71235	

The frequency distribution of these observations is given in Table II, where it is seen that the mode tends to lie between the bracket marked out by the 12,645 and 32,175 square micron limits. Graphic representation of this distribution is afforded by the dash line in the frequency polygon of Fig. 1.

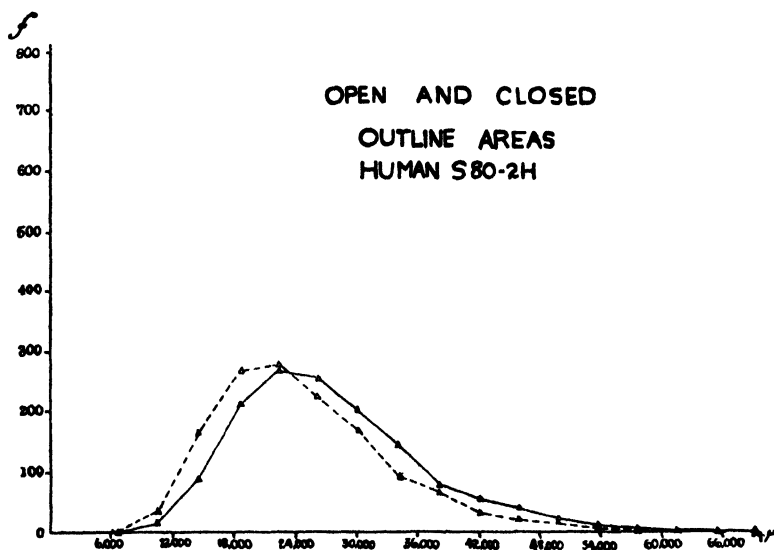


FIGURE 1.—Frequency polygon, constructed from data of Tables II and IV. Dash line—open outlines; Solid line—closed outlines. Ordinates represent the number of observations; Abscissae, the values of the areas in square microns.

Planimetric determinations from closed outlines. The closed outlines used for the AM4 diametric determinations in the same lung were measured planimetrically, and the detailed and grand averages appear in Table III, together with the standard deviation and probable error for the total lung of the mean closed outline areas. From this it will be seen that the grand average area of the 1,400 closed outlines is 27,549 square microns, or about 0.028 square millimetres. The lobar averages here again show the same trend as compared with the AM4 average diameters, for the smallest values are found in the lower lobes. As with the unidimensional results, these bidimensional values are higher for the closed than for the open outlines. This is further evidence that the greatest alveolar dimensions lie in planes that do not pass through the alveolar mouth.

TABLE III
HUMAN S80-2H AM4 PLANIMETRIC

All figures denote square microns

	Group	Right upper	Right middle	Right lower	Cardiac	Left upper	Left middle	Left lower	Average
AREA OF CLOSED ALVEOLAR OUTLINES	1st 100	30003	30084	23895	25054	27840	28203	26235	27330
	2nd 100	31387	29038	23785	24058	30826	28507	26769	27767
	Total 200	30695	29561	23840	24555	29333	28355	26502	27549

Standard deviation and probable error of mean closed outline area (total lung): 9159.6 and 165.11 square microns.

Table IV shows the frequency distribution of the data given in Table III. In the frequency polygon of Fig. 1 the areal values for the closed outlines are plotted on the solid line, and they may be compared with those for the open outlines, shown by the dash line.

TABLE IV
AREA OF CLOSED OUTLINES
HUMAN S80-2H

Class units in square microns	Number of observations
928 - 4833	
4834 - 8739	
8740 - 12645	17
12646 - 16551	86
16552 - 20457	210
20458 - 24363	266
24364 - 28269	252
28270 - 32175	201
32176 - 36081	145
36082 - 39987	81
39988 - 43893	55
43894 - 47799	40
47800 - 51705	22
51706 - 55611	13
55612 - 59517	8
59518 - 63423	2
63424 - 67329	1
67330 - 71235	1

Combined grand average of the planimetric results for open and closed outlines. A simple average of the final figures for the areal

determinations of both open and closed outlines is *26,231 square microns* or about 0.026 square millimetres. As for the similar combined average in the case of the diameters (1), it is felt that this combined average is a more representative index of alveolar size expressed as an area of a plane than is the grand average for either the open or closed outlines taken singly, for random cross sections of alveoli theoretically in every conceivable plane are thus represented, and there are twice as many measurements included—2,800 rather than 1,400. It is felt that this value is a reliable expression of human alveolar size in areal terms. It can, moreover, be used in the computation of alveolar capacity and wall surface (4).

Areal calculations from unidimensional determinations. A method for calculating the areas of open and closed outlines from linear measurements by methods AM1a and AM4 respectively has been devised utilizing the formula for computing the area of an ellipse (3). Applied to the diameter values obtained by AM1a the formula

$$\pi \frac{a.b}{4}$$

gives *26,410 square microns* for the average area of the *open* alveolar outlines. Using the AM4 averages the formula

$$\pi \frac{X.Y}{4}$$

gives *28,182 square microns* for the *closed* outlines. The combined average of these is *27,300 square microns* and this is within the experimental error of the similar planimetric figure of *26,231 square microns*. A reasonable approximation of the area of random alveolar sections is therefore afforded by calculations based on AM1a and AM4 linear measurements.

Discussion. The practical applications of these bidimensional results are similar to those already indicated for the linear dimensions obtained by methods AM1, AM1a, and AM4 (3). Areal determinations have the advantage of taking into account all irregularities, extensions, and prolongations of the alveolar outlines which sometimes are not taken into account because one or both diameters may not pass into such outpouchings. The planimetric determinations are made with relative ease, and the time factor is not appreciably greater than that involved in unidimensional methods where several diameters must be drawn and individually measured. These determinations of area also afford the most accurate basis for the computation of alveolar capacity and wall surface (4). It is interesting to note that areal calculations based on the unidimensional values agree with the actual planimetric measurements within the limit of experimental error.

SUMMARY

(1) The size of human lung alveoli has been expressed as an average of areas of alveolar planes which are indicated as open and closed outlines in 25μ sections.

(2) Using a planimeter and the same camera lucida tracings of the alveolar outlines as were used for the diameter determinations, the areas of 2,800 such planes were measured. The grand average of 1,400 open outlines was found to be *24,913 square microns* (about 0.025 square millimetres), and that of 1,400 closed outlines, *27,549 square microns* (about 0.028 square millimetres). The combined average of these figures is *26,231 square microns* (about 0.026 square millimetres). This combined average figure is regarded as a more representative value of alveolar size in terms of the areas of planes passing through the alveoli than is either the open or closed grand average alone.

(3) By a method of calculation from the diameters arrived at for the open outlines (AM1a) and for the closed (AM4) the combined grand average for the areas of planes through the alveoli was found to be *27,300 square microns* (0.027 square millimetres), and this is fairly close to the corresponding value obtained by actual measurement.

(4) As with the diametric results it was found that the greatest alveolar areal dimensions are in planes that do not pass through the alveolar mouth.

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WHY DID ONTARIO SALMON DISAPPEAR?

By A. G. HUNTSMAN, F.R.S.C.

THE salmon of the streams tributary to Lake Ontario (Fig. 1) disappeared during the latter part of the last century. There seems to be no definite record of any being taken in this century, although Nash (1908, p. 62) stated that "an occasional specimen is taken in Lake Ontario by the fisherman, but these visitors are probably merely wanderers from the hatcheries below." His explanation was probably based upon captures in the St. Lawrence River, such as the following. *Rod and Gun in Canada* reported in August, 1901 (3 (3): 20) as an extraordinary occurrence that a salmon had just then been speared in the St. Lawrence at Cornwall. In 1913, C. W. Young (1913, p. 39) wrote "within the past dozen years two or three fish supposed to be salmon were caught at Cornwall and Morrisburg. The only one seen by the writer had already been cut up for food and the head and tail destroyed, but it was undoubtedly a small Atlantic salmon. During the past summer several undoubted salmon (grilse) of seven or eight pounds weight were killed in the creeks above Brockville and exhibited in the market in that city." These fish were doubtless related to the salmon rivers discharging from the south into the St. Lawrence below Prescott. They cannot safely be attributed to Lake Ontario.

The latest record of the capture of an Atlantic salmon in the lake seems to be that of a 7-pound fish taken in a trout net about five miles off Scarborough Beach by William Montgomery about April, 1898, and identified by the editor of *Forest and Stream* (statement by Mr. John Townson to Professor J. R. Dymond). The latest record of salmon seen in a stream tributary to the lake seems to be that of a pair of 7 to 8 pound fish seen in Wilnot Creek in 1896 by Mr. A. W. McLeod of the Ontario Department of Game and Fisheries, then at the New-castle Hatchery. There had been fully 20 fish, some of them grilse, in 1893.

If the reasons for this disappearance were known, it might help either to prevent their disappearance elsewhere, or to bring them back to Ontario streams, or to increase their abundance anywhere.

HISTORY OF THEIR DECLINE

Although no records of the catches are available, it seems to have been clearly established that the numbers of salmon declined steadily through the middle of the last century, reaching a very low ebb in the

sixties. In 1869, in a special report Whitcher and Venning (1870, p. 66) stated for Wilmot's Creek near Newcastle as follows:

In early times it was famous for salmon, great numbers of which fish frequented it every autumn for the purpose of spawning. They were so plentiful forty years ago, that men killed them with clubs and pitchforks—women seined them with flannel petticoats—and settlers bought and paid for farms and built houses from the sale of salmon. Later they were taken by nets and spears, over one thousand being often caught in the course of one night. Their yearly decreasing numbers at length succumbed to the destruction practised upon them each season from the time of entering the creek, until nearly the last straggler had been speared, netted or killed. Such is, in short, an epitome of the history of every once populous water connected with Lake Ontario.

Samuel Wilmot, who developed the Dominion Fish Hatchery at Newcastle on Wilmot Creek, had knowledge of the change in that creek from the beginning of settlement until approximate extinction of the salmon. This was partly from his own experience and partly from that of his father, a native of New Brunswick who settled in 1816 on their farm through which the creek ran, selecting it because the creek "was at certain seasons of the year literally swarming with salmon, almost crowding themselves in certain 'runs' on the banks of the stream. The place was then almost an unbroken wilderness, and the Indians caught the fish in vast numbers" (Wilmot, 1882, p. 37). When Wilmot began to hatch salmon in the fall of 1866, these fish had become "so scarce as to make it a matter of great difficulty to obtain a sufficient number of parent fish" to carry out his plans for artificially "replenishing the stream" (Wilmot, 1868, p. 85).

After the hatchery was established, the numbers of the salmon increased from year to year, almost without exception, until an independent observer felt able to state:

no year has passed without my having often visited the stream during the spawning season, and have been delighted to see the marked increase of adult salmon which have year after year entered it, and I am satisfied I do not exaggerate when I say that in October last (1878) there were at one time, between the Government Fish House and the lake, a distance of less than two miles, three thousand salmon weighing from three to twenty pounds each. I would further state that from information I have received from persons living in other parts of Ontario, that there is hardly a stream between Brighton and Hamilton into which more or less salmon did not come during last autumn [Robson in Wilmot, 1882, p. 38].

This seemed conclusive proof that protection and artificial hatching could restore the salmon stock to its pristine abundance.

Unfortunately for this idea, in the year following this abundance, namely in 1879, there was a very small run, and in 1880 instead of a

recovery there was a further drop in numbers. This discouragement resulted in abandonment of attempts to restore abundance of salmon, since in 1883 they were able to obtain only 84,000 eggs as compared with 1,500,000 in 1876.

Salmon disappeared at different times from the various streams. For the Don River at Toronto, the last seen by Mr. John Smith, who owned a farm at the mouth of the river, entered in 1852 (statement to Professor Dymond by Mr. John Townson). Mr. John H. Taylor, grandson of the man who established Taylor's Mills on the Don after coming to Toronto in 1826, remembered looking for salmon in the Don and having them to eat in the early eighteen-sixties (statement in 1936 to Professor Dymond). Mr. C. W. Nash, Provincial Biologist at Toronto, told the Toronto Field Naturalists' Club in 1924 that the last salmon in the Don had been speared with a pitchfork under the dam by Mr. Taylor at Taylor's Mills "40 or 45 years ago," which would mean perhaps when the salmon again became plentiful in the lake in the eighteen-seventies.

When the Department of Fisheries attempted to bring back the salmon in the eighteen-seventies, it ignored the Don, but set aside for special protection the Humber and Credit Rivers and the Oakville and Bronte Creeks, since salmon were known to enter them at least occasionally. Of these the best was the Credit, which lacked entering salmon only in 1872 and 1877 of the years of special report and contained several hundred in the best years. In the Oakville Creek, they were reported as seen in 1878 (the best year) only. In the Humber, it was reported only that one large one was observed in 1872, one was caught in the spring of 1876, and some in the spring of 1878. The reports for Bronte Creek were less definite, salmon merely being stated to have been seen there in four different years, although this stream was nearest to the overseer's (J. W. Kerr) home in Hamilton. It would seem that salmon had by the late eighteen-sixties at least ceased to be regular inhabitants of all the streams from Toronto westward, although surviving in streams to the east.

Similarly, on the south side of the lake where they were equally abundant and generally distributed in the early days, Edmunds on investigating in 1872 found the salmon remaining only in Salmon River at the south-eastern corner (Smith, 1892, pp. 199, 200). In 1879, Kumlien (Goode, 1884, pp. 473, 474) found them as having been in fair numbers several years before and as being distributed from Oswego to Cape Vincent. In 1891, Smith (1892, p. 200) was able to learn only of a few recent captures of salmon—several of 2 or 3 pounds about

three years before along the shore near Oswego, one of 12 pounds on a fly rod in 1890 below the first dam in the Oswego River, and one of 7½ pounds in a gill net about August 17, 1891 in the Bay of Quinte.

SIZE, RATE OF GROWTH, AND SEXUAL MATURITY

Ontario salmon reached in some cases a weight of 40 pounds, one of that size being reported for Duffin Creek in 1874 (Kerr, 1875, p. 153) and Smith reported (1892, p. 196) that one of 42 pounds had been taken in the Salmon River in former times. The fish as caught in the lake were as small as 1½ pounds in weight (Kerr, 1878, p. 41; Smith, 1892, p. 197) and apparently fish as small as that returned to the streams, as Kerr (1878, p. 41) stated that two about 13 inches long were taken in the Credit River, one of them on a fly.

Information on the rate of growth, which can be determined from the scales, has had to be limited to the few museum specimens that have been available in recent years. Blair (1938) found that two specimens had had two years of stream life before becoming smolts. It would be expected from comparison with streams in the Maritime Provinces that salmon in Ontario streams would for the most part become smolts after two years' growth, but that some in the upper and cooler waters of a stream would remain for a third year.

Blair found that the two fish had spawned the next year after becoming smolts, that is, as grilse. This corresponds with the fact that small fish, that is, grilse were taken in the streams. When the salmon at Wilnot Creek were becoming more abundant in 1868, Wilnot (1869, p. 86) found them to be mainly grilse of from 2½ to 3 pounds weight and about 22 inches long, among 150 of which only three were females. Correlatively, when the salmon were decreasing rapidly in numbers in 1880 and 1881 (Wilnot, 1882, p. 12) they were practically all large fish and invariably females. This shows clearly that the sexes differed markedly in size and age at maturity, the condition resembling that for the St. John and Miramichi Rivers of New Brunswick, and contrasting with that for the Apple and Moser Rivers of Nova Scotia, where nearly all the females as well as the males return as grilse.

In correspondence with the females not spawning until the second year after becoming smolts, Wilnot (1871, p. 274) found that spawning salmon which he marked at Grafton Creek in 1868 did not appear there in 1869 but did appear ("many of them") in 1870. Similarly, salmon marked at Wilnot's Creek in 1871 were found among those returning in 1873, 27 so marked being found among 51 entering during one night (Wilnot 1874, p. 117).

The rate of growth is similar to that found in sea-running fish, not equal to the best, but at least equal to the poorest. In a river such as the Margaree, grilse run in weight from $1\frac{1}{2}$ to 6 pounds and two-sea-year-fish from 7 to 16 pounds, although smaller are known. The age or size at maturity and the maximum size resemble what is found for such rivers as the Miramichi or St. John.

MIGRATIONS

To settle the question as to whether or not Ontario salmon migrated to the sea, Wilmot (1869, p. 87) marked by cutting off the adipose fin all the salmon and grilse trapped at Grafton Creek in the fall of 1868. If they did so migrate, he expected that some of the marked fish would be taken below Quebec by fishermen. There is no record that any were so taken although many of the marked fish were re-taken at Grafton Creek in 1870. If any had entered Wilmot Creek, Wilmot would surely have mentioned the fact, which he does not. Similarly, the fish marked in Wilmot Creek in 1871, were apparently re-taken only in that creek.

On examination of the scales of Ontario salmon, Blair (1938) came to the conclusion from the character of the growth that these fish had never been to sea, finding that the growth was similar to that of the salmon of Lake St. John, Quebec, and significantly different from that of Miramichi salmon.

There seems to be no evidence that, when salmon were abundant in streams discharging into Lake Ontario, they were also abundant in the upper part of the St. Lawrence River, although Bonnycastle (in Fox, 1930, p. 46) in 1841 saw salmon being speared near the Cedar Rapids, thirty-two miles above Montreal. In those days salmon were doubtless migrating through the St. Lawrence between the lakes along the river or even the estuary and the Chateaugay, St. Regis, Racquet, Grass, and Oswegatchie Rivers of New York State, which empty into the St. Lawrence from Lachine to Prescott and which, according to Edmunds (in Smith, 1892, p. 199), formerly contained salmon.

When salmon became rather abundant in parts of the lake in the eighteen-seventies and an effort was being made to get information on increase in salmon numbers as being evidence of the success of fish culture at Newcastle, nothing was reported from the upper St. Lawrence. If the thousands of fish then found in Ontario streams had ascended from the Gulf of St. Lawrence, they would not have gone up the long stretch of river unnoticed, with so many fishermen eager to take such fish.

If the mature salmon found each season in Lake Ontario and its tributary streams had fed in the ocean or Gulf of St. Lawrence and had migrated up the St. Lawrence, they would not have been taken so early. The salmon of the gulf do not begin to migrate before May, which agrees with the lateness of the warming of the water there. In the Humber River the salmon appeared in April (Wilmot, 1872, p. 79, and Fox, 1930, p. 48) and "the earliest recorded date in the year for the capture of salmon is March 17, on which day in each year the Honorable Charles Small of Toronto served a lake or a river salmon at his table on the occasion of his daughter's birthday" (Fox, 1930, p. 50).

The local abundance of the salmon in the eighteen-seventies and the recorded results of efforts that happened to be made at that time to discover where they were still to be found provide a picture of the extent of their wanderings in the lake. It may be that these wanderings were conditioned by difficulty in entering their native streams owing to sand and gravel bars cast up by storms at their mouths.

On the north shore, the salmon seem to have persisted in a number of streams from Grafton Creek at the east to Duffin Creek or even the Rouge River at the west and they became rather numerous in this area. As has already been mentioned, they appeared in certain years in smaller or larger numbers in streams to the west at least as far as Bronte. There was somewhat remunerative netting permitted in the lake near Newcastle and Cobourg. Some 200 salmon from 6 to 15 pounds in weight were taken in nets set near Newcastle in 1871 (Wilmot, 1872, p. 84), those taken in May before arrival of salmon from Quebec selling for 50 cents per pound. In 1875, nets set near Newcastle took 120 salmon during the latter part of July (Wilmot, 1876, p. 24). In 1876 in three weeks' fishing, three nets at Cobourg took about 100 salmon and the nets at Newcastle took 240 from 8 to 18 pounds in weight, 28 fish in one day (Wilmot, 1877, p. 368). In 1877, 143 salmon were taken at Newcastle and some at Cobourg (Wilmot, 1878, p. 21). Traps were not permitted elsewhere, so that records are confined to captures made with gear operated to catch other fish. Such reports began to indicate an increase of salmon in 1874, with 5 salmon taken in hauling seines (Kerr, 1875, p. 152). Six are given for 1875, at Toronto, Four Mile Creek, and Burlington Beach. For 1876, there were 8 in all, taken at Frenchman Bay, Rouge River, Burlington Beach, and Grimsby (Kerr, 1877, p. 332). For 1877, there were 17, taken at Frenchman Bay, Rouge River, Toronto, Burlington Beach, Burlington Bay, and Winona (Kerr, 1878, p. 46). For 1878, there were 23

fish, taken at Frenchman Bay, Port Credit, Bronte, Burlington, Dundas Marsh, Winona, and Grimsby (Kerr, 1879, p. 330 and 363). For 1879, there were "quite a number" (Kerr, 1880, p. 327), and for 1880, there were 9 (Kerr, 1881, p. 299). For 1881, there were "two large salmon in prime condition taken one near Winona and the other at Queenston in the Niagara River," also several others elsewhere (Kerr, 1882, p. 253). These records show an increase and then a decrease in numbers and a somewhat progressively more extended distribution around the western end of the lake. It may be that the salmon reported as taken at Wilson, New York State, twelve miles east of the Niagara River in 1879 (Smith, 1892, p. 197) represent a further extension of this distribution.

The above described spreading of the stock of salmon from the rivers east of Toronto is in the same direction as the general movement of the lake water. Evidence is lacking of any spreading in the opposite direction, that is, eastward from those rivers.

In 1872, the salmon on the New York side of the lake were restricted to Salmon River in the south-east corner (Edmunds in Smith, 1892, pp. 199-200). Evidently they became more abundant and more widely distributed afterwards, since in 1879 they are reported (Kumlien in Goode, 1884, pp. 473, 474) as having decreased in that river after 1875 and as being taken, at least a few, from Oswego to Cape Vincent. A decade or so later they are reported (Smith, 1892, p. 200) as so rare that there was failure to get definite knowledge of a single specimen being taken on the New York side of the lake in 1891, although one was taken in the Bay of Quinte that year and one in the Oswego River the previous year. The picture presented is similar to that for the north-west part of the lake—namely an increase in the seventies where they had survived, with spreading therefrom chiefly in the direction of circulation of the water around the lake, which is contra-clockwise.

CHARACTER OF THE BETTER SALMON STREAMS

The distribution of the salmon in Ontario streams was recorded by the Department of Fisheries in the eighteen-seventies, when it was attempting to restore them to abundance. Wilmot trapped salmon for fish-cultural purposes in Wilmot Creek (Newcastle), Grafton Creek, Duffin Creek (Pickering), and Barber Creek (Bowmanville), but not in Smith Creek, that is, the Ganaraska River (Port Hope) in spite of its nearness to his establishment and its large size. He also repeatedly described the natural spawning and sometimes gives details of the numbers of both salmon and spawning beds in the streams trapped for

breeding fish. Kerr, living in Hamilton, and with a district extending from Whitby to Niagara around the western end of the lake, frequently gave details of the numbers of salmon entering and the numbers of spawning beds made in Lynde (Lyon's or Lynd's) Creek (Whitby), Duffin Creek, Rouge River, and Highland Creek, e.g. for 1877 (1878, p. 41) 55 salmon and 40 beds in Duffin Creek, 12 salmon and 6 beds in Lyon's Creek, 5 beds in Little Rouge River and 3 beds in Big Rouge River. He reported salmon entering Humber River, Credit River, Sixteen Mile Creek (Oakville), and Twelve Mile Creek (Bronte), but failed to give any details of their spawning, and he made no mention of salmon even entering any streams in the remainder of his district.

A stream's value for salmon depends upon the success of spawning in producing smolts to continue the stock. There was quite general appreciation of the importance of assuring that salmon reached the spawning beds, but apparently not that the salmon needed to be well distributed up a stream. Attempts were made to keep the mouths of the streams open for the salmon at spawning time and on one occasion Duffin Creek had to be opened eight times, owing to storms on the lake blocking it (Kerr, 1877, p. 332). There were many dams on the streams, some of which the salmon could surmount, and some of which were stated to be impassable. Some effort was made to have fishways in dams that needed them for passage of the fish, but it is quite uncertain to what extent the salmon were able to ascend, although it is mentioned that in 1872 salmon were seen in the Rouge a long way up in the township of Markham (Wilmot, 1873, p. 101). That such far ascent might not be considered necessary is indicated by Overseer Kerr's recommendation for Lyon's Creek (1878, p. 41) that a wire gate should be put in it to prevent the salmon going farther up than the Kingston Road bridge because salmon were known to get frozen in the stream farther up. Wilmot was content to have the salmon spawn in a small stretch of the river below his hatchery, and this is perhaps explained by his comment on Duffin Creek (1870, p. 62) that "if salmon were allowed to pass beyond the mill up the stream . . . not one would escape destruction from the hands of the lawless among the inhabitants."

In the Maritime Provinces, our studies show a relation of salmon to streams or parts of streams with a rather steep slope and plenty of stones and gravel in the beds. These tend to be found cutting down through alluvial hills, the swift water of heavy freshets eroding the banks, carrying away the lighter and finer material and leaving in the bed of the stream the heavy and coarse material—gravel and stones—to be shifted and cleaned of accumulated debris by every new freshet.

Thus spawning beds are provided which have water percolating through them for aeration of the eggs, and also there is the rough rapid water with varied cover that the young frequent.

Examination of the contoured maps of the Topographical Survey shows that the Don River, from which the salmon disappeared early, has in its lower part a very low slope, taking eight or nine miles to reach 125 feet above lake level (Fig. 2). The Humber and Credit require only about five miles for such a rise, but the former takes about nine miles for the second 125 feet, whereas the latter takes only three miles or so. The order in the disappearance of salmon from these three streams seems to have corresponded with the way in which they are graded in degree of slope in their lower portions. In contrast with these streams, streams to the east (with the exception of the Ganaraska River), in which the salmon persisted longer, have steeper slopes in their lower portions. Main branches of the various streams rise the successive 125 feet heights above lake level in numbers of miles as follows: Highland, $3\frac{1}{2}$, $2\frac{3}{4}$ and $2\frac{3}{4}$, $2\frac{1}{4}$; Rouge, $3\frac{3}{4}$, 4 and $2\frac{3}{4}$, $2\frac{1}{4}$; Duffin, $4\frac{1}{4}$, $1\frac{7}{8}$ and $5\frac{1}{4}$, $3\frac{1}{4}$; Lynd, $4\frac{3}{4}$, $1\frac{3}{4}$ and $3\frac{1}{2}$, 3; Bowmanville, $1\frac{7}{8}$, $3\frac{1}{4}$; Wilmot $3\frac{1}{4}$, $2\frac{1}{2}$; Grafton, $1\frac{3}{4}$, 1.

Examination of some of the streams confirms the view that steep slope in these streams to the east is associated with a relatively large amount of clean, exposed gravel and stones. Highland Creek, Rouge River, and Duffin Creek have definitely more than the Don, Humber, and Credit Rivers or than the Ganaraska. In the Rouge the gravel is so abundant that it is being hauled away, doubtless for road use. It seems clear that streams that near their mouth cut down through alluvial hills containing stones of varied size were best for salmon production, at least latterly.

To the extreme west and south of the lake, the Niagara escarpment provides very steep slopes, but its rocky character gives impassable falls rather than gravelly and strong rapids. This doubtless is the reason why salmon were absent from streams of that part of the shore of the lake.

SEASON OF STREAM ASCENT

There is no suggestion in the reports that salmon ever entered the streams east of Toronto at any other time than the fall, that is, near and during the spawning season in October and November. Exceptionally a few might enter late in September. In contrast, both the Humber and the Credit Rivers are stated to have had both spring and fall runs of salmon (Wilmot, 1872, pp. 79, 80). Although evidently

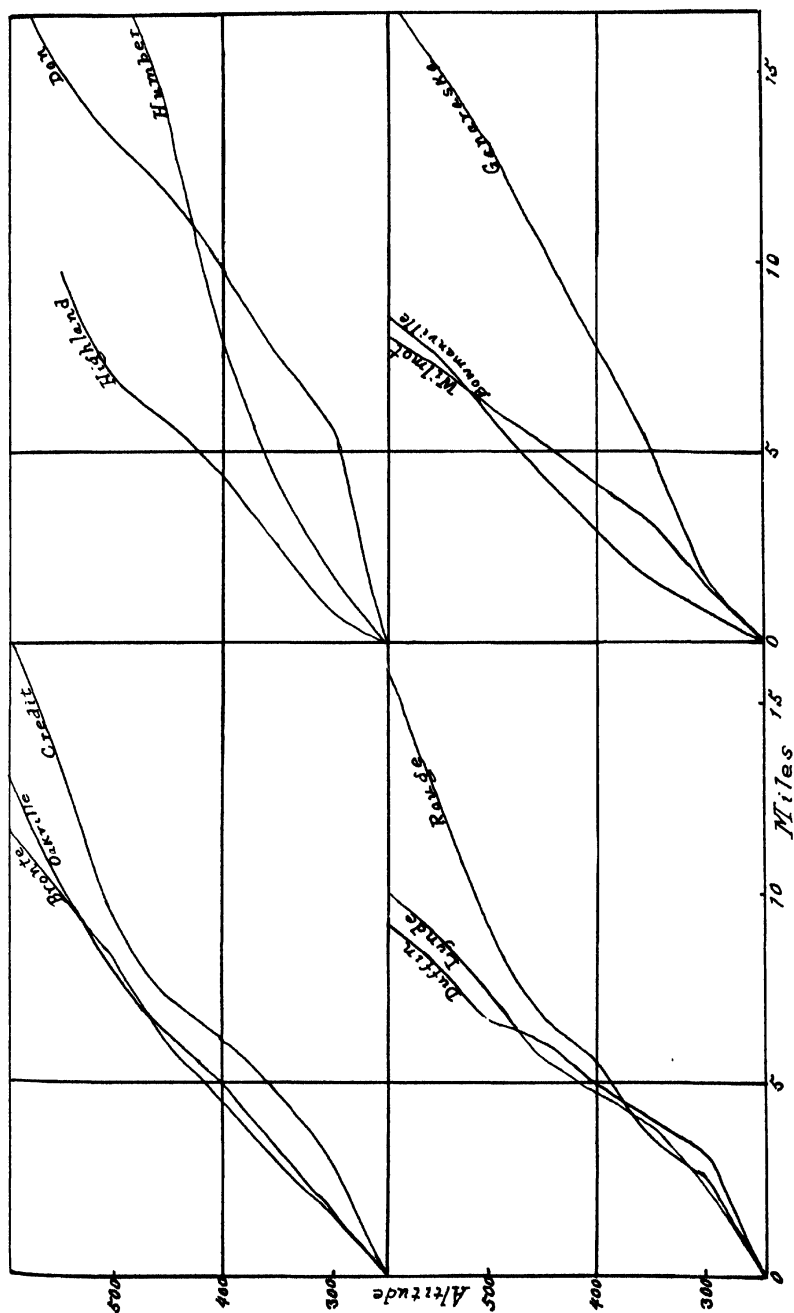


FIGURE 2.—Slopes of various streams discharging on the north shore of Lake Ontario. Altitude is given in feet above sea level as shown in the inch to the mile maps of the National Topographic Series of the Department of National Defence. Distances in miles are measured from 250 feet above sea level, that is slightly above the lake level.

not producing salmon in the eighteen-seventies, the Humber did have salmon entering it in three different years, for two of which (1876 and 1878) the time is given as the spring, and never as the fall, which distinguishes it from all the other streams, including the Credit.

Smith (1892, p. 195) states that "the fish approached the shores in June and . . . went up the streams to their head waters, deposited their spawn and returned again to the lake" evidently relying on reports made to him by fishermen. Such spring spawning has never been shown to be a characteristic of the Atlantic salmon and the report is doubtless erroneous. Those entering the Humber in the spring were stated (Wilmot, 1872, p. 79) to have been "bright and silvery in color, rich and fat in flesh, in prime condition" as compared with the fall salmon, which "were dark in color, lean and lank in flesh, out of condition, being at that season of the year engaged in the work of spawning." Clearly, the spring salmon of the Humber were comparable with the large fish that enter Maritime rivers in the spring, but do not spawn until fall.

The factors determining time of entrance are but vaguely known. The large fish run early and for 1872 it was indeed stated (Kerr, 1873, p. 145) "one large salmon was observed in the Humber River." Large streams and streams that discharge into bays open to the south tend to have fish that enter early, while small streams discharging on a straight coast have only late-running fish. The Humber and the Credit are the largest rivers of the district and the Humber in particular is associated with a bay at its mouth.

FLUCTUATION IN ABUNDANCE

A marked feature of the last decades of the Ontario salmon was a very rapid increase in numbers from 1869 to 1878 and a catastrophic decline from 1878 to 1880. A similar fluctuation in salmon abundance during that period characterized the whole Atlantic coast and was particularly pronounced in the Chignecto system (Huntsman, 1931, p. 16), of which the chief river is the Petitcodiac. This river resembles the Ontario streams in having a large part of its watershed devoted to agriculture.

Whatever background there may have been for this extreme fluctuation in salmon abundance, there must have been a factor or factors that varied during the period in such a way as first to increase salmon numbers and then to decrease them. A relation has been found between height of the water in the streams during the crucial summer months and later abundance of the adult salmon as shown in the records of

quantities taken by the nets (Huntsman, 1937, pp. 22, 23), and such a correlation may be sought for in this case.

There are no records of water heights covering the period in question. Records of rainfall give some indication of what the levels probably were, even though, as in this case, the records are confined to one place, which may be far from representative of the watershed. Fortunately this place, the only one for which records are available, was Toronto, which is at least on the fringe of the significant area. For the abundance of the fish we have the somewhat fragmentary records made by Wilmot of the numbers of salmon trapped in Wilmot Creek and the numbers of eggs laid down each year in the Newcastle hatchery. It will be seen (Fig. 3) that from 1841 to 1865 there was a rather steady, slow decline in the summer and autumn rainfall which was accentuated in the last eight years. This agrees with the reputed decline in salmon abundance, which reached a very low ebb in the late sixties, causing Wilmot to undertake cultural measures to bring it back. It should be realized that low water in the rivers may have acted on the young salmon at any time in the open period of the year after the spring floods, that is, from June to November, and, while affecting the spawning only in the late autumn, low water at that time would be more or less determined by the amount of rain that had fallen previously. For these reasons the rainfall for the summer and autumn has been plotted in Fig. 3, not only as a whole, but for each of the three successive two-month periods.

It should also be understood that the effect of low water on numbers of adult salmon is a delayed one, the length of the lag between cause and effect depending upon the length of life of the fish and the time in their life history when the cause operates. Low water may have affected spawning or survival of the young in their first or second years in the streams, and the adults may have been one-year-in-the-lake (grilse, and mostly males), two-year-in-the-lake (ordinary salmon and mostly females), or even older fish. The effect would, therefore, be expected to appear anywhere from two to five years after the rainfall and to persist for several years longer.

The high rainfall in 1866 may be considered to have been responsible for increasing abundance toward 1870, which received a setback from low rainfall in 1867 and 1868. High rainfall in 1869 and 1870 would then cause the upward surge in salmon numbers that began about 1874 and reached its peak in 1876. There followed six dry summers from 1871 to 1876, and those doubtless brought about the catastrophic decline from 1878 to 1880.

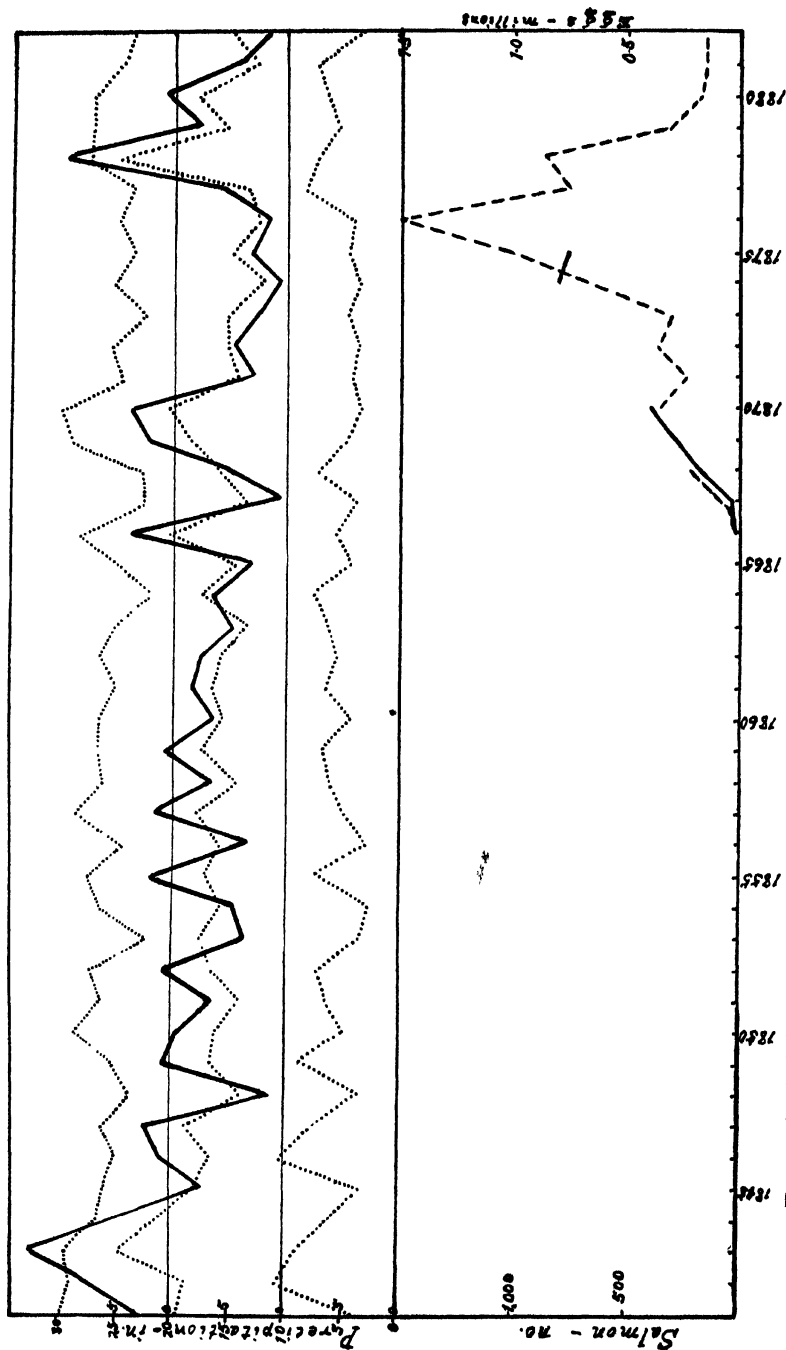


FIGURE 3.—Rainfall and abundance of Ontario salmon. Yearly rainfall above, from the official records for Toronto: Continuous line, June to November; dotted lines, June-July (upper), August-September (middle) and October-November (lower); Yearly amounts of salmon below: Continuous line, fish entering Wilnot Creek; interrupted line, eggs laid down in Newcastle Hatchery.

With this decline there was failure of hope of restoration of the salmon, and stoppage of records of their occurrence and numbers, so that any later effect of rainfall cannot be followed. So far as they go, the records support the conception that variable rainfall was an outstanding cause of fluctuation in abundance of Ontario salmon.

The lag between rainfall and numbers of salmon appears to have been from two to six years. Heavy rainfall in 1866 was followed in 1868 by an abrupt increase in numbers of grilse, but the numbers of salmon increased for at least another two years. The drop in rainfall from 1870 to 1874 was followed six years later by a corresponding drop in salmon abundance from 1876 to 1880.

CAUSES OF DISAPPEARANCE OF SALMON

When the stock of salmon had become very small, Wilmot (1869, p. 85) and Whitcher and Venning (1870, p. 66) gave reasons for the change and urged fish culture and protection to bring back abundance of the fish. Wilmot (1878, p. 16 and 1882, p. 40) gave with fuller experience a different explanation of the decline in numbers. When the stock was practically gone, Wright (1892, pp. 449 and 468) and Smith (1892, pp. 195, 201) gave expert opinion on the matter.

Overfishing. Wilmot (1869) at first blamed "the overfishing, the murder on the spawning grounds, the trap-net fishing along the shores of the lake and the estuaries of the streams, and the excessive demand and greed for the fish." Whitcher and Venning considered it to have been a factor but Wright thought it only a contributory cause. Smith stated that "the catching of salmon *per se* was not the cause of their decrease."

Starting in 1866, the Dominion Government by Order-in-Council, set aside certain streams, which were thought to have salmon still entering them, for natural and artificial reproduction. These streams were placed under special guardianship to prevent the fish being taken in any way. Apart from fish taken for cultural purposes, the only fishing for salmon that was permitted was a small amount of netting of salmon in the lake near Newcastle and Cobourg for several years as has already been described. The fish entered certain of the streams in the fall near spawning time and were carefully protected until they left these streams after spawning.

Nevertheless, the salmon, although increasing rapidly for some years, decreased in numbers even more rapidly while these measures continued to be carried out. Overfishing cannot have been the cause of the disappearance of these fish, and indeed salmon have such a high

power of reproduction that they continue under very intensive fishing. Only very thorough removal of the fish in the confined waters of the streams would eliminate the stock.

Removal of forests. Wilmot later (1882) came to the conclusion that "the change is therefore in the waters; and that change is due to the clearing of the forest off the land, in the neighbourhood of these streams and their feeders and the consequent reduction in water volume by reason of the increased evaporation." Wright expressed it as "the removal of forests . . . the resulting changes in rainfall, or at least in the extent to which surplus rainfall is held back by forest land and underbrush, and thus delivered only gradually and not in torrents through streams."

It has not yet been established that removal of forests of itself reduces the flow of streams as has been generally believed, however greatly other things usually associated with forest removal may do so. In addition, it is found that full forest along streams is associated with a sparse population of fish, which indicates that a certain amount of forest removal will increase salmon abundance.

More rapid drainage. Clearing of the land was usually followed by agriculture, which ordinarily involves good drainage. Wright mentioned "the reclaiming of swamps," and indeed land upon which water usually lies has to be thoroughly drained to make it suitable for farm crops. Undoubtedly ordinary agriculture involves a quick run-off. "The generally reduced flow of water in the streams" (Wilmot, 1885, p. 22), on which so many people with experience through the period of settlement of the land insist, is doubtless to be thus explained and is a very real condition.

The reduction in the volume of water in the streams during dry periods will undoubtedly lower their fish-carrying capacity, as the correlation found between summer discharge of a river and the fishery yield from its stock of salmon clearly shows (Huntsman, 1937). This does not, however, provide a reason for the disappearance of the salmon.

The quick run-off was considered inimical to the salmon—"immense freshets . . . carrying away previously formed spawning grounds" (Wilmot, 1878, p. 16). While it is conceivable that heavy freshets may do considerable damage to spawning beds, this is not at all certain. It is the streams with heavy freshets that produce the most young salmon, doubtless through the action of the freshets in clearing the gravel beds of debris and silt that impede movement of water and so smother the eggs. The South West Margaree River has but moderate changes in water flow because it is fed from the very large Lake Ainslie which

delivers the water but slowly, and this river in comparison with the lakeless North East Margaree or its own lakeless tributaries produces but few young salmon.

Clearing of the stream bed. Wilmot suggested (1878, p. 16) the loss of "the constantly splashing current against logs and fallen trees" that "gave both aeration and hiding places innumerable for the fish." He attributes the loss to the heavier freshets, but it seems more probable that it has been the result of man's clearing of the banks and bed of each stream. An unimpeded even flow, however desirable for other reasons, makes a comparative barrenness of fish. This might well have caused a decrease in salmon abundance, but not their disappearance.

Pollution of water. Whitcher and Venning stated that "manufactories and farming along the banks had fouled and changed the creek from its natural state and made it less capable of affording shelter and spawning grounds," and Wilmot (1882) wrote of "the defilement by the surcharge with vegetable matter, field-filth, rubbish and foul matter." Also Wright mentioned "The pollution of the streams by sawdust and other refuse."

The extent of pollution and its effects are difficult to gauge. The authorities of government have been more or less constantly on the alert to reduce or eliminate it. That the streams are found to support a varied and frequently abundant population of young fish requiring conditions similar to those suitable for young salmon is sufficient proof that pollution has not been sufficiently serious to cause the salmon to disappear.

Silting of spawning beds. As one of the causes that "had well-nigh exterminated the salmon from the waters of Ontario," Wilmot (1878, p. 16), from his experience in carrying salmon eggs in the water from Wilmot Creek, wrote "the foul particles of sediment permeate everywhere, covering the eggs at times during the course of a few hours, to the depth of half an inch with a muddy mixture of putrid earthy and vegetable matter." Wherever such a condition exists, there is no need of looking further for an explanation of the disappearance of the salmon.

A very large proportion of the land drained into the former salmon streams around lake Ontario came into cultivation during the course of the nineteenth century. The very great increase that cultivation makes in the amount of silt washed from the lands into the streams has been repeatedly demonstrated. Examination of varied streams in October and November of 1943 has shown their bottoms to be so silted up that in comparison with the successful salmon-spawning streams of the Maritimes, they fail to provide proper conditions for salmon redds.

This is particularly true of the lower portions of the streams, to which the spawning seems to have been restricted for some time before the salmon disappeared.

The cultivation of the land seems to have nearly reached its highest level about the eighteen-sixties, while the salmon did not altogether disappear until the nineties. It seems probable that the silting was a cumulative process to some extent and that the amount of silt in the streams continued to increase. Further study may settle this point. There is also a possible relation between the silting up of the spawning beds and rainfall as to the amount of silt produced and as to its settling in or on the beds.

Prevention of salmon ascent. Wright stated that the disappearance of the salmon was unquestionably due to the drying-up and the pollution of the streams and "to obstacles like mill-dams preventing the ascent of the fish toward the head-waters." Smith quotes Commissioner McDonald: "The cause of the disappearance . . . has been chiefly and primarily the erection of obstructions in all the rivers, which have prevented the salmon from reaching their spawning grounds." Wilmot, although mentioning the significance of dams in stopping ascent of salmon, did not definitely blame them for the decrease in salmon abundance. He did state (1873, p. 102) that "in many of the creeks running into Lake Ontario a serious drawback to the reproduction of the salmon is occasioned by the annual decrease of the volume of the water running through them" which acted by the weaker current failing to keep a passage open through the "long, narrow, gravelly beach" "formed by the action of the lake water during rough weather" "at the mouths of these creeks," so that "salmon, in many instances, are unable to enter these streams" and "are therefore compelled to lay their eggs upon the gravelly sandy shores of the lake." He found also that with low water the salmon, even after entering the stream might largely fail to ascend to his "reception house," at least the larger fish, so that "from the fishery buildings down to the end of the rapid part . . . about a mile and a half . . . was literally dug up by the salmon wherever gravel could be found upon which to form a nest. On one occasion, within the distance of one hundred rods in the stream, one hundred and twenty freshly worked beds were counted" (Wilmot, 1874, p. 116). The reality of the blocking of the mouths of the streams is shown by the statement by Kerr (1877, p. 332) for Duffin Creek in 1876 that "the mouth of the creek was stopped up so often by lake storms, that the guardian had to open it eight different times during the breeding season."

It seems unquestionable that the barriers to ascent of the salmon to spawning grounds, whether in the form of dams, of bars at the stream mouths, or of shallows in the streams, were definitely responsible for marked decreases in the salmon stock. This factor doubtless became rather steadily more accentuated during settlement of the country. It may be questioned, however, whether it can be considered as responsible for the disappearance of the salmon, which occurred after the land was approximately fully settled, when dams were becoming fewer and when quick run-off was probably not being much accentuated. At any rate, the presence of many dams and the decreased flow in periods of drought provided a very poor background for continuance of the salmon stock.

REMEDY

If prevention of access of salmon to spawning beds and silting up of the beds were causing them to disappear, hatching the eggs artificially and planting the young should have been the appropriate remedy. It was attempted by Wilmot and failed. Why it failed is a question that needs to be answered, but we have few details of his operations, and we cannot yet be said to know how much hatching and planting should be carried out to assure the desired result. To bring back the salmon to streams entering Lake Ontario by regularly planting the young therein may prove well worth-while. One thing seems certain,—that success will depend upon carefully following the outcome of experimental planting so that we may discover how to produce the most adult salmon with the least effort.

If the beds are silted up, measures to ensure access of the adult salmon to the streams will not give successful natural reproduction. It would be necessary to have deep, clean gravel beds. If soil erosion were stopped, freshets would in the course of time produce such beds, and any made artificially would not be rapidly silted up and rendered useless.

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THE EFFECTS OF ASPHYXIA, CARBON DIOXIDE, AND ETHER ON THE pH OF THE BLOOD

By R. A. WAUD

Presented by C. C. MACKLIN, F.R.S.C.

THE pH of the blood is controlled by three factors: (1) the buffer systems of the blood; (2) the excretion of fixed acids by the kidney; (3) the excretion of the carbon dioxide by the lung. The last factor is the one with which this study is mainly concerned.

By means of a glass electrode connected in the circulation of the dog estimations of the pH of the blood were made almost continuously at intervals of 10 seconds or more. Both Heparin and "Liquoid" were used as an anticoagulant.

In some experiments, in addition to recording the hydrogen-ion concentration of the blood, the respiratory rate and volume were recorded by means of a recording spirometer. The following were studied; the effects of asphyxia due to clamping the trachea and decompression, the administration of oxygen, carbon dioxide, and ether. Pentobarbital was used as a basal anaesthetic.

When the trachea was clamped there was an average fall in the pH of the blood of approximately 0.18. This increase in acidity was obtained in less than one minute and was maintained until the clamp was released. This increased acidity is no doubt due to an increase in partial pressure of carbon dioxide in the blood resulting from the increase in the lung. Following the return to normal there was a slight rise above normal and then a return to normal. The results of a typical experiment are represented graphically in Fig. 1.

The administration of 100 per cent oxygen caused only a slight fall in the pH of the blood.

The administration of 2 per cent carbon dioxide in pure oxygen caused some lowering of the pH while 15 per cent carbon dioxide had a marked effect. This usually amounted to approximately 0.3. In one experiment, however, the change amounted to 0.42 and was maintained as long as the carbon dioxide was administered, but on its removal the pH returned to normal in one minute and then rose slightly above. The results of the administration of 15 per cent CO₂ with pure oxygen are shown graphically in Fig. 2.

Placing the animal in a rarefied atmosphere to simulate an altitude of 30,000 feet caused a rise in pH which followed fairly closely the

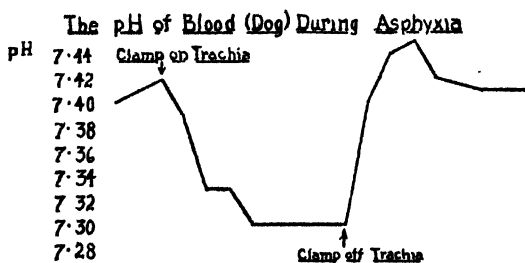


FIGURE 1.—Changes in the pH of the blood of the dog on clamping off the trachea.

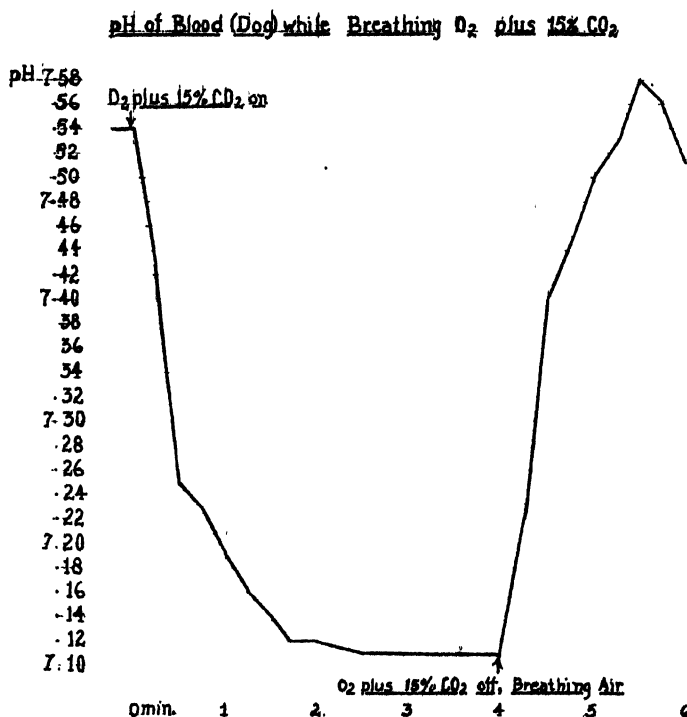


FIGURE 2.—Changes in the pH of the blood of the dog on the administration of 15 per cent carbon dioxide.

process of decompression. The increased alkalinity, however, was not maintained as the animal was held in the rarefied atmosphere but soon began to fall. When the animal was recompressed this fall extended an equal distance below the normal. The rise in pH accompanying decompression is probably due to decreased partial pressure of carbon dioxide resulting from hyperventilation and decreased atmospheric pressure. The reverse of this takes place on recompression. No explanation is offered for the fall in pH during later part of the decompressed period. In some animals the pH of the blood did not

The pH of Blood (Dog) During Decrease of Pressure
to a Stimulated altitude of 30,000 feet.

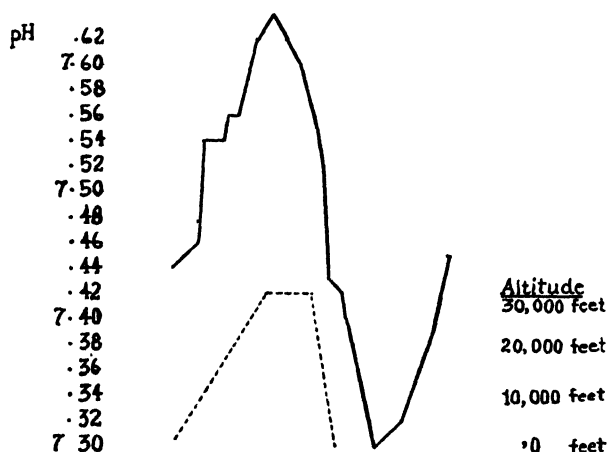


FIGURE 3.—Changes in the pH of the blood of the dog on decompression to a degree equal to 30,000 feet.

follow this above pattern. A satisfactory explanation of the variation has not been found. The results of a typical experiment are shown graphically in Fig. 3.

When ether was administered a marked fall in the pH was observed. This effect was very constant and was usually accompanied by a marked fall in respiratory volume. This would suggest that these changes in pH are due to decreased elimination of CO_2 from the blood. When the reflexes set up by irritant vapour coming in contact with the mucosa of the respiratory tract become dulled such as obtains in deep anaesthesia, other factors may be at work. This phase has not been

investigated. As would be expected any relationship between the respiratory rate and the pH exists only in so far as the former changes the respiratory volume. Fig. 4 is a graphic representation of the results of a typical experiment.

The advantages of this *in vivo* method of estimation of the pH of the blood are evident. The rapidity with which determinations

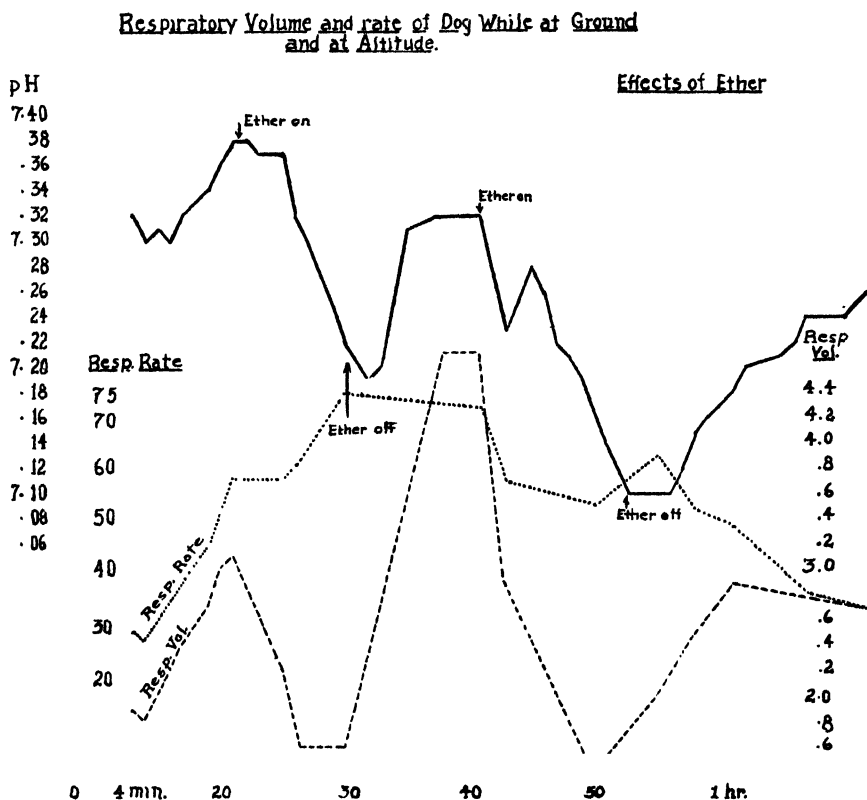


FIGURE 4.—Changes in the pH, respiratory rate, and volume, of the blood of the dog on the administration of ether.

can be made—practically continuously—gives a more accurate picture of the changes in the blood than would be possible with methods where it is necessary to remove the blood from the circulation. In this connection it has been amply demonstrated throughout this work, that the pH of the blood is not constant but varies over periods of a few

seconds. It appears that the most important factors in bringing about these rapid changes are the changes in the activity of the animal which in turn results in variations in the production of CO_2 . To this is added the response of the respiratory centre to these variations in CO_2 content of the blood. An additional advantage is that any changes due to contact of the blood with the air or other manipulation are avoided. The disadvantages are that the method is applicable only in acute experiments in animals; an anticoagulant is necessary; and the technique is somewhat difficult.

SUMMARY

(1) When the CO_2 content of the lung is raised by asphyxia, the administration of CO_2 or ether, the pH of the blood is lowered.

(2) Decompression causes a rise in the pH followed by a fall.

(3) Rapid and small but definite changes are almost constantly taking place in the pH of the blood of an animal the activity which is at all variable.

Thanks are due Mr. Wm. Bending for his excellent technical assistance in this work.

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SECTION V

SERIES III

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NORTHERN LIMITS OF WHEAT PRODUCTION¹

By ROBERT NEWTON, F.R.S.C.

WHEAT production in Canada is mainly confined to the southern plains region of the Prairie Provinces. Its possible northward extension concerns not only the potential world food supply, but also the feasibility of northern settlement. The opening of the Alaska Highway and other northern developments have stimulated new interest in the local food resources of those regions.

My association with the University of Alberta, which, though the most northerly situated university in Canada, is still south of the geographical centre of the province, led to my special interest in the northward extension of wheat-growing. Investigations of one kind or another, bearing on this question and extending over the past quarter-century, form the basis of this address.

Every good husbandman knows that success in crop production depends in the first instance on choosing crops and varieties which are well adapted to local climatic conditions. We do not expect in northern Alberta to compete with Illinois and Iowa in growing corn and soybeans, but we regularly excel them in the yield and quality of the common cereals. But even for cool-season crops a severe limit is set by the length of the frost-free season.

In his *Northward Course of Empire*, a book published in 1922, Stefansson puts forward the theory (in which he found later he had been anticipated by Macoun) that from some point in the United States where killing summer night frosts first become a hazard, their

¹Contribution from the University of Alberta and the National Research Council of Canada. Published as Paper No. 242 of the Associate Committee on Grain Research.

Apart from the material in the section on industrial qualities of wheat, the results presented have not hitherto been published, except for short notes on the controlled experiments in annual reports of the National Research Council. I am much indebted to research students and colleagues for the progress that has been made. Among those who carried on particular studies or assembled and analysed data are Dr. J. A. Anderson, Dr. W. H. Cook, Dr. J. W. Hopkins, Mr. W. R. Jack, Mr. C. Kenway, Dr. J. G. Malloch, Dr. J. B. Marshall, and Dr. A. G. McCalla.

frequency would increase northward to a certain point, but after that decrease as we proceed further north, because of the decreasing length of the summer nights. In the 1941 *Yearbook* of the United States Department of Agriculture (p. 213), Stefansson writes: "At Lacombe, near the southern boundary of Canada, during 15 years the average frost-free period was only 69 days; but at Beaverlodge, 200 miles farther north, it was 80 days and at Fort Vermilion, 230 miles farther north than Beaverlodge, it was 88 days."

But other figures based on official records I have looked up, while they do not invalidate this theory, at least appear to weaken it. During the seventeen years ending 1938, the mean frost-free periods for the three places mentioned by Stefansson deviate in part from his order as follows: Lacombe 77, Beaverlodge 94, and Fort Vermilion 78. Moreover the periods in individual years ranged from 49 to 101 at Lacombe, 64 to 124 at Beaverlodge, and 44 to 104 at Fort Vermilion. In some years the order of the three places was the reverse of that required by the theory. Again, other Alberta points included in the table I consulted did not fall in the order required by the theory with respect to the first three. For example, Athabaska, in a latitude not very different from that of Beaverlodge, had a mean frost-free period of 60 days as compared with 94 at Beaverlodge. While, therefore, lengthening days and shortening nights may and should diminish the danger of night frosts under a given set of conditions, it is clear that the effect of other factors may often predominate. Altitude is of course one such factor.

GROWTH PERIOD OF WHEAT IN RELATION TO LATITUDE

The real significance of the long summer days in northern latitudes, as a factor in wheat growth, is of great interest. Plants occupy their unique position as the ultimate source of the food supply of the world by virtue of their power to transform the radiant energy of the sun into chemical energy in the form of carbohydrates and proteins. Increased light periods, other things being equal, in addition to any indirect effect through modification of frost hazards, would be expected to accelerate growth and shorten the season required to produce a given yield per acre. Moreover, the progressive development of a plant from stage to stage through its life cycle is favoured by progressive changes in ratio of day to night. The further north we go, the more rapidly do the days lengthen following the spring equinox. Consequently, the more rapidly should the wheat plants receive photo-periodic stimuli to progressive development. Do these apparently

favourable light conditions actually hasten the growth and maturity of wheat as we go northward?

In 1924-5, with the co-operation of the superintendents of a number of experimental stations, I collected data on the length of the growing period of four wheat varieties at fourteen stations in the Great Plains, ranging from Hays, Kansas, latitude 39° north, to Fort Vermilion, latitude $58\frac{1}{2}^{\circ}$ north, for the seventeen seasons 1908 to 1924 inclusive. Though none of the stations grew all four varieties every year, the data were numerous enough to justify some comparisons. The average results, when plotted, showed that all four varieties reacted the same way, but did not show a gradual northward shortening of the period from seeding to maturity, as would be expected if light were the only factor involved. The graph (Fig. 1) was actually very irregular but, on the whole, showed a slight lengthening of the growing period northward. Obviously other factors, such as moisture and temperature, modified the results. Long-dash broken lines are used in Fig. 1 to bridge gaps caused by the absence of a given variety at certain stations.

Kincer (1919) put forward the theory that active growth does not begin in the spring until the mean daily temperature reaches 43°F . He based this on the fact that the average daily fluctuation in spring and fall, in most parts of the United States, is about 20°F ., and on the assumption that as long as the night temperatures drop to the freezing point not much growth takes place. The average daily fluctuation increases northward, but we used Kincer's value to add in Fig. 1 the short-dash broken lines which purport to correct the growing season of the varieties concerned by dating these from the beginning of the active growing season as defined by his theory.

It is common knowledge that the crops from early sowings do not mature proportionately early in the fall. The late Sir Charles Saunders used to reckon ten days' delay in seeding made about five days' delay in ripening, and so on in that proportion of about two to one. Photo-periodism is one influence in this relation, as I mentioned a moment ago, but temperature also plays its part. Cool weather in early spring retards growth. Moisture may in some cases be the determining factor. The surface soil often dries in early spring to a point where germination of seeds fails to take place. Then the seed merely lies inert until the next rain comes. Thus early sowing may for a variety of reasons add fictitiously to the apparent length of season required by a given crop to mature.

On the other hand, Kincer's theory fails to take account of the fact that the first part of the life of an annual plant takes place below the

surface of the soil, where daily temperature fluctuations are less, and the mean temperature is higher. Thus, while the "corrected" lines placed on Fig. 1 do smooth out some irregularities which may be partly fictitious, as just explained, direct observations by persons in charge of the stations concerned show that active growth did in fact begin before the dates based on Kincer's theory. In other words, the corrections are too great, and the true lengths of growing period of these wheat varieties are not as short as the dotted lines indicate.

With respect to stations in the open plains of the United States, there is a well marked pattern in the fluctuations of the original curves, which seems clearly related to altitude. Beginning with Hays, Kansas, as we proceed from station to station, a shift westward (and consequently a rise in altitude) is accompanied by an increase in the length of the growth period required, while a shift eastward produces the opposite effect. This is the more notable since a shift westward means moving to an area with less rainfall, a change which should shorten the growth period if no other factor supervened. In this case the effect of altitude seems to predominate.

A. D. Hopkins (1918) proposed as a bioclimatic law for temperate North America that, other conditions being equal, the variation in time of occurrence of a given periodical event in life activity is at the general average rate of four days to each one degree of latitude, five degrees of longitude, and four hundred feet of altitude, *later* northward, eastward, and upward in spring, and the reverse in autumn. J. W. Hopkins (1938) translated this into terms of monthly mean air temperatures for eighteen years at forty-three points in the southern halves (essentially the well settled halves) of Saskatchewan and Alberta. He found that during the period May to August inclusive the mean temperature was about 0.3°F. cooler for each 10' north latitude, 0.05°F. warmer for each 10' west longitude, and 0.55°F. cooler for each one hundred feet of altitude. This helps to explain the predominant effect of altitude noted above.

CONTROLLED EXPERIMENTS

It seemed probable that at high altitudes with colder nights there would be a period of time, both morning and evening, when the light might be quite sufficient for photosynthesis but the temperature too low. The same seemed likely to be true of northern latitudes. This led to the decision to investigate under controlled conditions the interaction of light with temperature, and their threshold values for the growth of wheat plants.

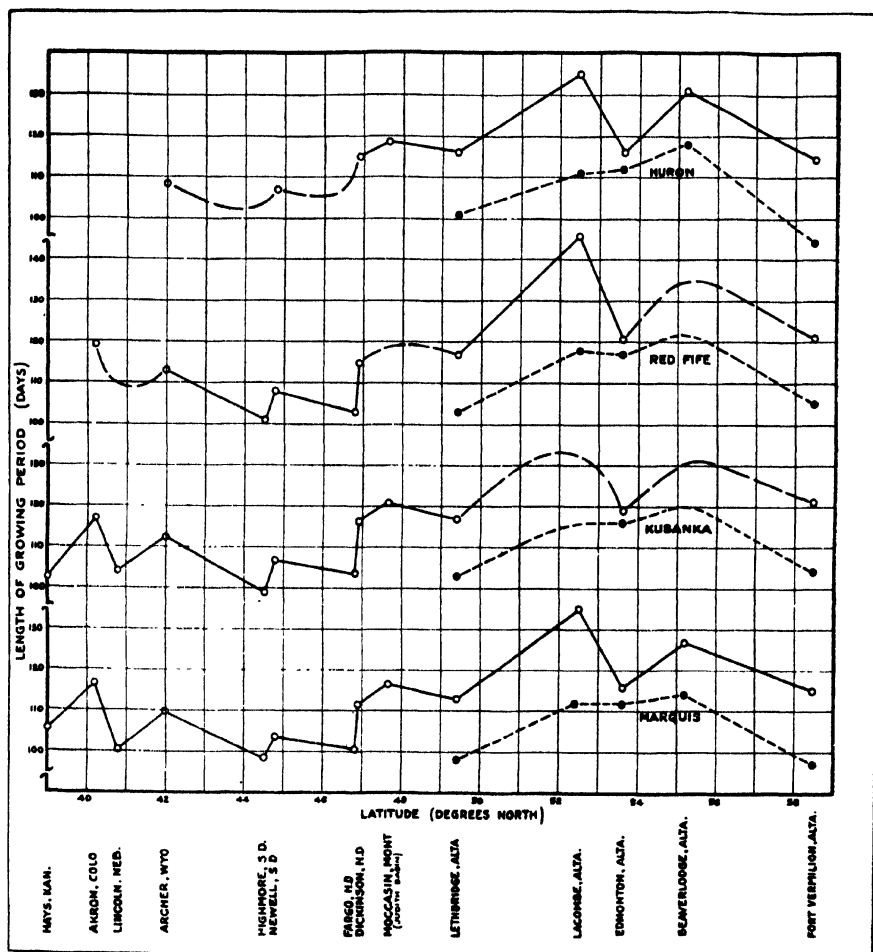


FIGURE 1.—Growth period of four wheat varieties in relation to latitude.

Plant physiologists have long cherished the ideal of controlling their plant-growth experiments with the same precision that characterizes well executed experiments in physics and chemistry. Unfortunately, it is very expensive to do this on any large scale. In the equipment we built in the National Research Laboratories at Ottawa (for designing and standardizing which Dr. W. H. Cook took main responsibility), we contented ourselves with floor space enough in each cabinet to accommodate twenty-five mason jars, half-gallon size, and head room sufficient for four weeks' vigorous growth, starting with seedlings a week old. Plate I shows wheat plants grown for this period at 65°F. and 1800 foot candles. A pair of these chambers was built, of which short descriptions have been published (Newton and Cook, 1932-3). Though several years of gradual development and improvement passed before we felt ready to begin applied experiments, we did in the end achieve satisfactory control of light, temperature, and humidity. Nutritive conditions were controlled by the simple expedient of changing the culture solutions at frequent intervals. A special germinator for producing uniform seedlings was developed with almost equal care.

One interesting point brought to light by the preliminary experiments is that plants are more sensitive to variations in physical factors of the environment than were the expensive physical instruments we used to measure these factors. With all our care to secure uniform conditions, we still found it necessary to rotate the positions of individual jars three times a week through a series of predetermined randomized arrangements.

The culture jars rested on a multiperforate platform through which the 2 m.p.h. current of conditioned air passed up around them, which platforms could be lowered gradually during the course of an experiment, by chain and sprockets moving on four threaded posts supporting the corners of the platform, in order to keep the middle of the plants an approximately constant mean distance from the light source at the top. The bank of mazda lamps used was separated from the growth chamber by water of controlled temperature flowing over a glass plate. The lights were automatically controlled by electric clocks, and were on sixteen hours each day.

In any given four-week run, relative humidity and temperature were the same in both chambers, only the light intensity being varied. As the current of conditioned air passing through the chambers was exhausted into the small room containing both of them, the plants were not exposed to any important change of conditions when the chambers were opened to rotate the position of jars, or to change culture solutions, or to weigh and measure the plants.

Two series of experiments were carried out, one in 1938 by W. R. Jack, the other in 1940 by Dr. J. B. Marshall, who assembled the data from both. The 1938 series included three temperatures: 45, 55, and 65°F.; and four light intensities: 200, 600, 1200, and 1800 foot candles. The 1940 series extended this light range to include 2400 and 3000 foot candles and added a fourth temperature, namely 75°F. Four gaps were left in the possible combinations of these light and temperature conditions, two of these by omitting 45° with 2400 and 3000 foot candles. The heat load thrown into the system by the bank of mazda lamps at these high light intensities made it impracticable to maintain a constant temperature of 45°. It was subsequently decided to omit also the other extreme combinations, i.e., 75°F. with 200 and 600 foot candles.

A selected strain of Marquis wheat was used throughout the experiments. Seedlings selected for uniformity of height and weight were placed in perforated waxed corks, four seedlings to a jar, and packed with non-absorbent cotton. In the 1938 series, measurements were made weekly on all of the plants, but only the total fresh weights of four plants including the corks were recorded at the weekly intervals, as none of the plants were harvested till the end of the fourth week. In 1940, one plant from each jar was harvested weekly, partly to increase the range of data recorded and partly to prevent the larger plants produced at the higher temperature and light intensities from crowding the chamber and so interfering with light distribution and air flow. The usual measurements of leaves and roots, number of tillers, etc., were recorded for this plant, with additional data on fresh and dry weights of tops and roots. Transpiration losses from the jars were made up with distilled water and recorded.

The very large number of data accumulated have been examined statistically, and may perhaps form the basis of more technical papers when the pressure of war duties is relieved. Meanwhile it will be enough to outline here the general trends shown by the actual results.

Seedling weight and plant weight

Not all the precautions one can take in controlling the environment can rule out the inherent variability of individual plants. In the present study, one hundred seedlings of height 60 ± 5 mm. were selected from a much larger population, and the weight of each seedling recorded. In 1938, the seedlings were selected by height only, and their weights ranged from 0.13 to 0.21 gram. In 1940, they were selected by both height and weight, the range of weight being restricted to 0.03

gram. The height of the seedling did not appear to have any consistent relation to its weight, nor to the weight of the plant after four weeks. But heavier seedlings produced heavier plants, though not as heavy as would be assumed from a uniform relative growth rate. However, it was obviously desirable to adjust final plant weights to the average seedling weight (0.1651 gram) in comparing the effects of experimental conditions, and this was done.

Total growth

Light and temperature effects are not mutually exclusive. The two factors co-operate in promoting growth, as may be seen in Fig. 2, where

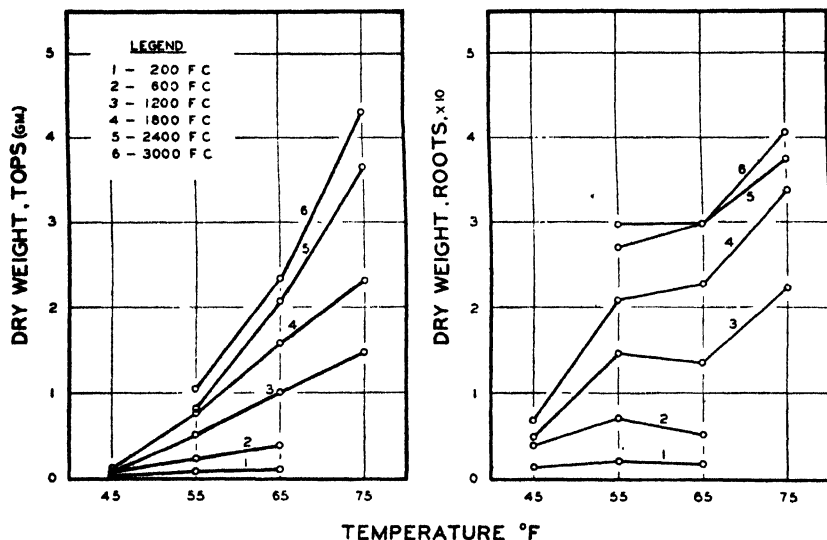


FIGURE 2.—Dry weights at twenty-eight days, of tops and roots at various light intensities, in relation to temperature. Note that vertical scale is ten times greater for roots.

the growth curve for each successive light intensity lies above its predecessor and, for tops at least, all move regularly upward with temperature. That is, an addition of either heat or light means more growth. True, the effect of added heat at the two lowest light levels is so small as to be almost negligible and, what is more important to our original thesis, we see in Fig. 3 that the addition of light at the lowest temperature has also a negligible effect on growth. There seems no doubt, therefore, that much of the daylight in northern latitudes is wasted for lack of sufficient heat.

Another deduction from Fig. 2 is that at 3000 f.c. the law of diminishing returns is beginning to operate, the curve for this light intensity being closer to the curve for 2400 f.c. than the latter is to 1800 f.c. This supports the common opinion that about one-third full sunlight is enough for maximum growth. Full summer sunlight at Washington, D.C., at air mass 1, is equal to 10,000 f.c. On the other hand, Fig. 3 suggests that the temperature maximum has not yet been reached.

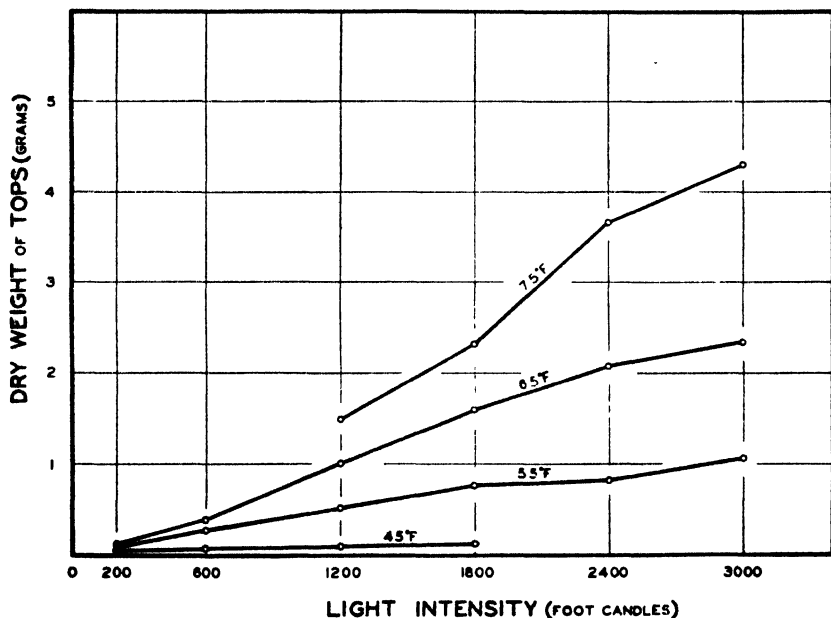


FIGURE 3.—Dry weights at twenty-eight days, of tops at various temperatures, in relation to light intensity.

Looking at Fig. 4 we get a slightly different impression. Here the mean relative growth rates for twenty-eight days in terms of per cent increase per day in the fresh weights of entire plants, are plotted against light intensity. Successive temperature increases become progressively less efficient from this point of view, and the advantage of extra light with 65°F. practically disappears at 1800 f.c. and with 75°F. at 2400 f.c. These seem to be, as we shall see again later, points where light and heat are in good balance.

Figs. 5a and 5b show the mean weekly relative growth rates of entire plants, fresh weight basis, plotted against time. We see that at 45°F. there was an initial lag, the maximum rate being reached in

the third week, and that the maximum was raised by each increment of light. At the lowest light there was an initial decline, possibly a result of transferring the seedlings from the germinator where in all cases they were germinated at 65°F. (with ample light sixteen hours daily) for the week preceding their introduction to the growth chambers. At 55°F. all but the 2400 and 3000 f.c. groups had their maxi-

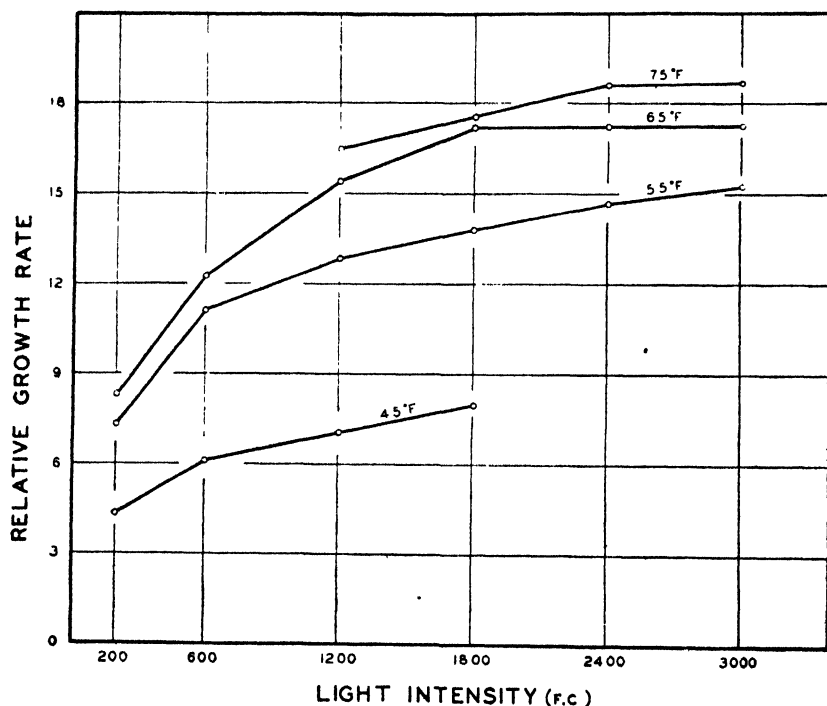
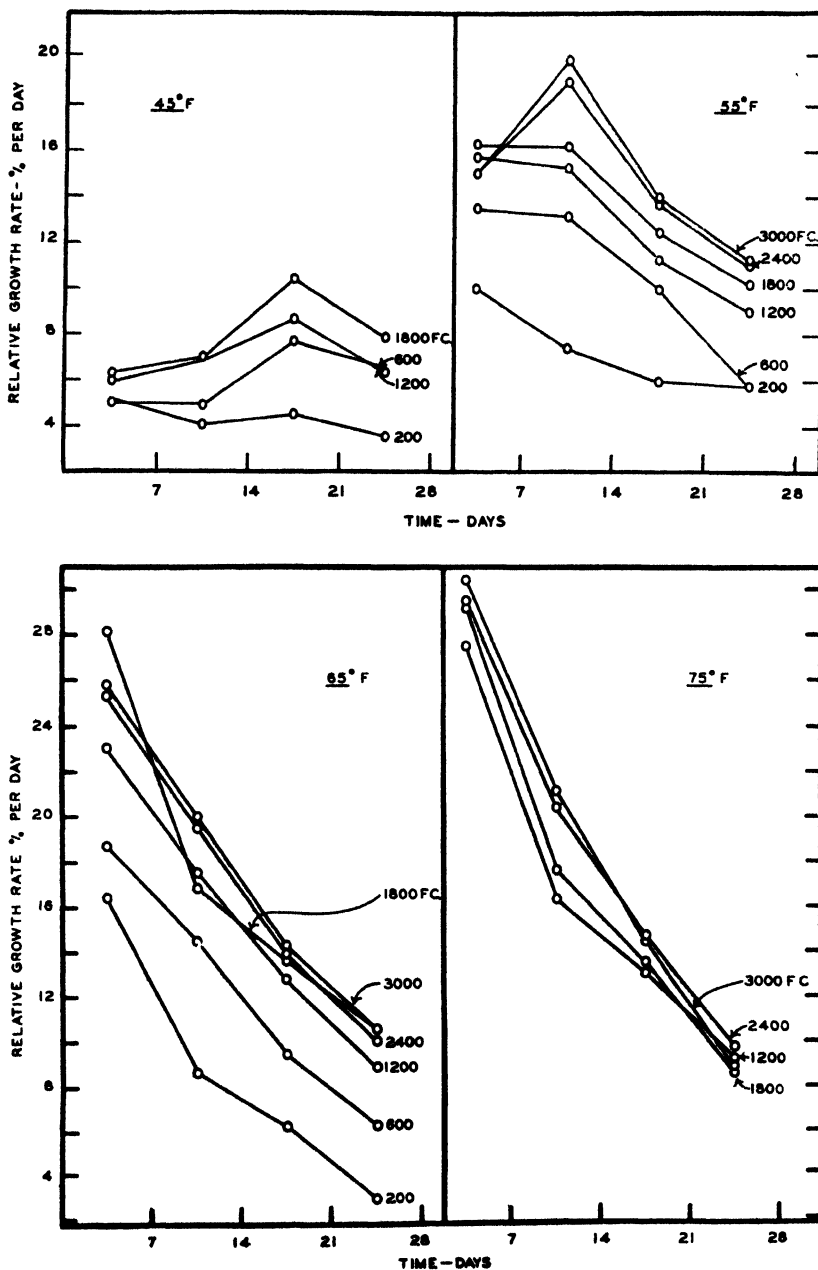


FIGURE 4.—Mean relative growth rates for twenty-eight days of entire plants, fresh weight, at various temperatures, in relation to light intensity.

mum growth rate in the first week. As the temperature was raised, the rate of decline from the initial week's rate became steeper. The general conclusion from these two figures is that the relative growth rate (though not the rate of increase in actual weight as shown in Fig. 2) diminishes practically from the earliest stages of development, and that the more rapidly the plant passes from stage to stage, the more steeply the growth rate falls off.



FIGURES 5a and b.—Mean weekly relative growth rates of entire plants, fresh weight, at various temperatures and light intensities.

Morphological development

Light and temperature conditions may affect not only the rate of growth but also the morphological development of plants. Table I shows the ratios of tops to roots found in our experiments.

TABLE I

RATIOS OF DRY WEIGHTS OF TOPS AND ROOTS AT TWENTY-EIGHT DAYS

Temp. °F.	Light intensity, f.c.					
	200	600	1200	1800	2400	3000
75	—	—	6.64	7.03	9.79	10.80
65	6.51	7.58	7.56	6.99	6.95	7.89
55	4.36	3.81	3.50	3.64	3.04	3.58
45	2.84	1.86	1.74	1.73	—	—

It is obvious that the top: root ratio increased with temperature, but this does not necessarily indicate an important differential effect on the growth rates of tops and roots. It probably reflects mainly the differences in stage of development reached in twenty-eight days at the various temperatures. The relative growth rates of both roots and tops decrease with time, but the root growth rates fall more rapidly. In other words, the roots develop earlier and the proportion of tops increases later. Light effects are less evident in Table I, except at the two extremes. A combination either of low light with low heat or of high light with high heat seems to favour the tops. The latter combination so accelerated development that in four weeks root growth was tapering off, while the tops were still growing actively. The superficially similar effect at the low light, low heat extreme may be only the abnormal outcome of such wide departure from optimal conditions.

The main effect, then, of adding light or heat was to accelerate growth, and so to advance the stage of maturity and increase the amount of growth in any given time. The number, length, and surface area of leaves, the number and length of tillers, and the length of the roots naturally increased in general with the stage or quantity of growth. But there were certain specific and well marked morphological effects.

Fig. 6 shows that the height of the main culm increased with temperature up to 65°F. No further increase was produced by 75°F. until light of 2400 or 3000 f.c. was associated with it. This suggests,

as we might expect, that light and heat must be in reasonable balance in order to get the best results from both. The number of leaves fully emerged (indicated by figures on curves) was, however, greater in all cases at 75°F., thus assuring greater production even if the plants at the two lower light intensities were slightly shorter than at 65°F.

The addition of light at any temperature caused a gradual increase

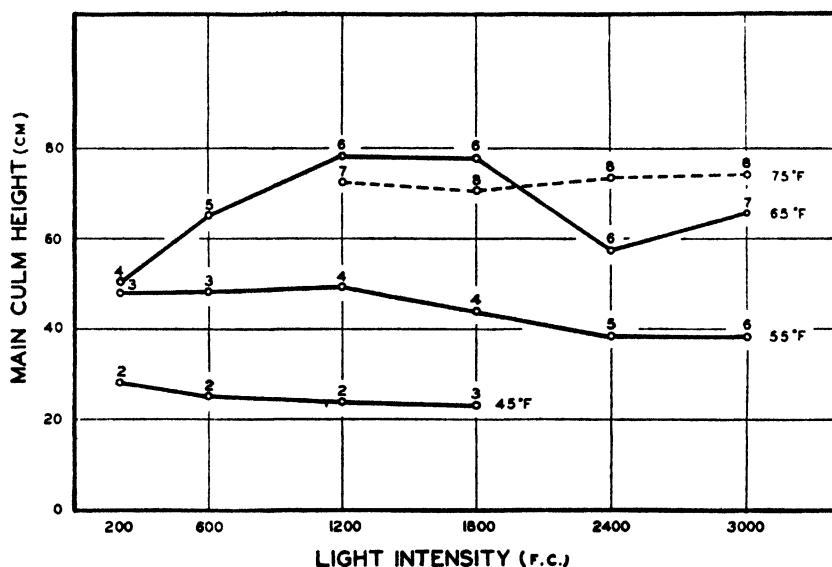


FIGURE 6.—Height of main culm, after twenty-eight days, at various temperatures, in relation to light intensity. Figures on curves indicate number of leaves.

in the number of leaves until a maximum of eight was reached. But the addition of light, except in the early part of the 65° series, did not cause any increase in the height of the main culm. Indeed, it tended rather to depress it. The spindling, drawn-out growth resulting from abnormally low light is well known. The initial rise in the 65° curve probably reflects the favourable reaction of the plants to the correction of the extreme unbalance of heat and light represented by the first two points. Why it should drop sharply at 2400 f.c. is not explained unless it be fortuitous.

Absorptions of ions

Since all the plants were grown in precisely the same nutrient solutions, the tops and roots were analysed to see whether any effect of light and temperature on the absorption of ions could be detected.

Calcium, magnesium, phosphorus, potassium, manganese, boron, and iron were determined either by chemical or spectrochemical methods, according to the quantity of material available and the abundance or scarcity of the elements. Only the first four showed definite trends, and only for tops was an entirely complete series of analyses by one method (chemical) obtained. The latter data are assembled in Table II.

TABLE II

PER CENT ASH CONSTITUENTS IN DRIED TOPS OF TWENTY-EGHT DAY PLANTS

Temp. °F.	Light intensity, f.c.					
	200	600	1200	1800	2400	3000
Calcium						
75	—	—	.473	.422	.302	.284
65	.835	.608	.469	.435	.308	.298
55	.664	.606	.500	.484	.396	.392
45	.574	.569	.502	.438	— —	—
Magnesium						
75	—	—	.203	.196	.125	.130
65	.415	.324	.206	.182	.150	.139
55	.373	.369	.201	.154	.164	.156
45	.329	.285	.237	.212	—	—
Phosphorus						
75	—	—	.657	.546	.482	.398
65	1.045	.725	.695	.581	.513	.472
55	.868	.770	.762	.532	.552	.654
45	.757	.859	.755	.662	—	— —
Potassium						
75	—	—	5.32	4.72	3.72	3.12
65	9.36	7.38	7.70	6.59	4.32	4.14
55	8.31	8.40	4.99	4.07	4.75	4.60
45	7.55	5.61	4.35	4.38	—	—

Again we find that the changes in content of these elements largely reflect the progressive stage of growth of the plants. But here light effects appear more prominently than temperature effects, the reverse of the situation we found in top : root ratios. One would expect heat to accelerate the absorption of ions from the culture solutions, and this

is borne out by the first column of the table, but as the light intensity rises it apparently accelerates carbon assimilation more than enough to overbalance mineral absorption, since the gradient in the columns of the table reverses as we move to the right.

Nitrogen content of the plants at twenty-eight days was also determined, because it is the critical factor in the industrial quality of wheat grain. But the only obvious deduction from Fig. 7 is that nitrogen followed its usual course of decreasing in the plants with their stage of growth which, as we have already seen, was in general more advanced by each addition of heat and light. As all culture solutions were maintained at the same concentration, the foregoing result is not surprising.

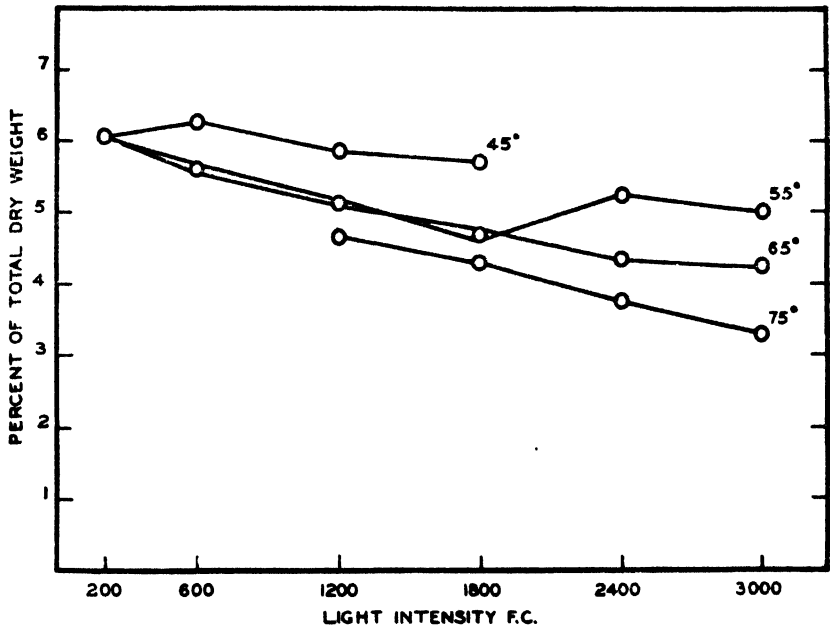


FIGURE 7.—Nitrogen content of entire wheat plants at twenty-eight days.

Water requirement

Use of water is one of the best measures of efficiency of growth, as affected by environmental factors. In Fig. 8 we see that any serious deficiency in light and heat or any lack of balance between them increased the quantity of water required to produce each gram of dry substance. The origin of the 45° and 55° curves represents deficiency in both factors. The origins of the 65° and 75° curves represent un-

balance. The low points in each curve may be assumed to represent the best balance, and these correspond roughly with maximum relative growth rates as seen in Fig. 4. It is noteworthy that the use of water increased with temperatures above 55°F., even though growth was more rapid and, except at lowest light intensities, appeared to visual observation more efficient.

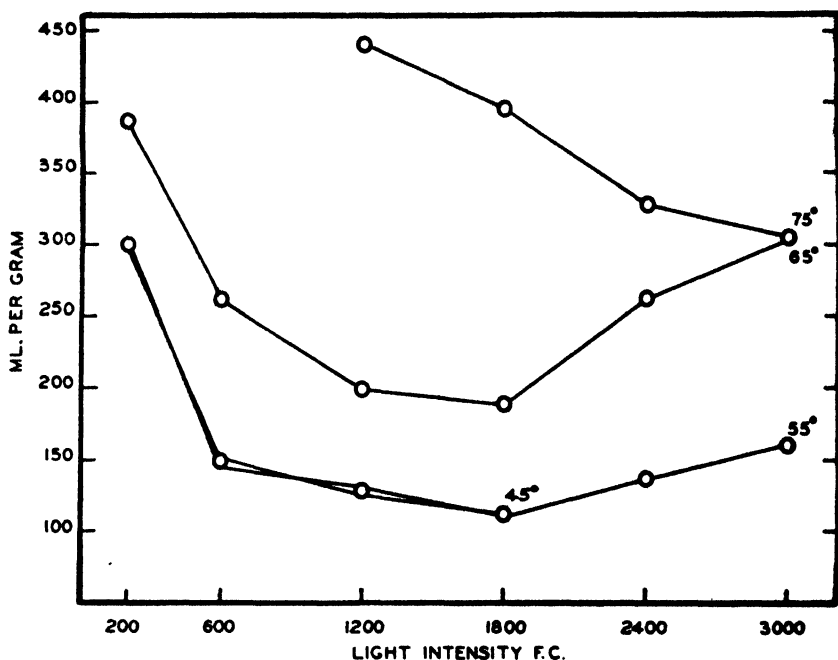


FIGURE 8.—Water requirements of entire wheat plants for twenty-eight days.

General observations

The plants were grown in half-gallon jars painted black to exclude light and then with white enamel to reflect both light and infra-red rays. At the end of each experiment, a cork containing four plants (in the 1938 series) or one plant (in the 1940 series) was transferred to a clear jar of water for photographing. Plate II shows the photos in the 1938 series. The lowest horizontal group provides ocular proof of our earlier conclusion that the addition of light at 45°F. is not very profitable, and establishes our thesis that the failure of longer days in north latitudes to hasten the maturity of wheat may be attributed primarily to the cool mornings and evenings. The left-hand vertical

group demonstrates our other conclusion that the addition of heat in the absence of sufficient light is also unprofitable. This, however, is of scientific rather than of practical interest from the point of view of these experiments. At 55° we already see substantial profit for each increment of light, and at 65°, as we can verify by looking again at Plate I, a light intensity of 1800 f.c. promises a bumper crop.

INDUSTRIAL QUALITIES

Having grown our wheat, what can we do with it? Wheat is unique in the possession of the gluten proteins, which cause wheat flour, on admixture of water, to form an elastic dough. A lump of bread dough, by the installation of a suitable gas generator such as yeast, can be blown up into a sort of pneumatic honeycomb which, on the subsequent application of heat, sets into the object we call a loaf of bread. Canada is world famous for the strong bread-making qualities of her wheat, and wheat has long been, and will doubtless continue to be, one of our leading exports.

But this reputation was built originally on wheat produced in the southern plains of the Prairie Provinces. As settlement pushed northward into the park belt with its fertile black soil, it was found that yields were larger, but breadmaking qualities poorer in the sense that the wheat had less reserve strength with which to bolster up the weak European wheats with which it was commonly blended. Plant breeders worked hard, and with considerable success, to produce early ripening varieties which were naturally high in protein content and of good milling quality. But as production pushed still further north into the gray, wooded soils they found themselves fighting a losing battle. Those regions produce excellent oats, barley, flax, clovers, and grasses, but were not designed by nature for industrial production of wheat. The most that should be attempted there under present conditions is to grow wheat for local use. Wheat is too vital a part of our commerce to risk losing any part of our market by offering a low quality product.

Two years ago I presented to Section V of the Royal Society of Canada for Dr. J. A. Anderson, Chief Chemist of the Board of Grain Commissioners, a paper entitled, "Causes of Geographic Variations in the Protein Content of Western Canadian Wheat." From the collection of lantern slides used then (Anderson and Eva, 1943a) I have borrowed three to remind you that the major soil zones of the Prairie Provinces correspond with zones of natural vegetation and climate, and these in turn with wheat yield and protein content. The southern prairies

have brown soil, with short grass cover, the lowest rainfall, the highest summer temperatures, the lowest wheat yield, but the highest protein content and best quality. The black parkland soils which half encircle the brown soils on their northern periphery are characterized by long grass and poplar bluffs; they are high in soil nitrogen and with adequate rainfall give good wheat yields of moderate protein content. The gray, wooded soils which in turn skirt the black soils are low in nitrogen and, though ample rainfall brings good yields, the wheat is generally low in protein and of inferior milling and baking quality.

Two figures taken from another paper from the same laboratory (Anderson and Eva, 1943b) illustrate the protein content of corresponding grades of wheat drawn from the northern and southern portions of the Prairie Provinces. In all cases the distribution curve for the protein content of carlots from the northern area is shifted to the low protein side of the diagram.

Finally, Dr. Anderson has supplied a new slide showing the distribution of carlot shipments over 0.5 per cent protein ranges for each of the past five crop years. In every year a few carlots fall to 10 per cent protein and in some years 15 or 20 per cent of all shipments may fall to 12.5 per cent or less protein content. It is a moot point whether any wheat of less than 12.5 per cent protein should be allowed in the export grades.

Dr. A. G. McCalla, Professor of Plant Science in the University of Alberta, has supplied figures for some half-dozen points in the gray soil belt of Alberta covering various periods up to thirteen years, which show that it is not uncommon for wheat on gray soils to fall to 10 per cent protein, and occasionally to less than 9 per cent. True, the Soils Department of the same university has shown that by proper crop rotation and manuring practices it is possible to raise these wheat protein levels substantially, perhaps enough to make the product quite satisfactory for home use. But it seems doubtful that it would be sound policy to attempt to develop the gray soils as a source of commercial wheat. It would appear wiser to exploit their undoubted suitability for mixed farming, with prime quality bacon, dairy products, flax, and forage crop seeds as their distinctive products.

In 1928 and 1929 I was concerned with inquiries on this continent (Newton and Malloch, 1929) and in Europe (Newton, 1930) as to the feasibility and desirability of making protein content a factor in the grading of wheat. The conclusion then was that the difficulties were greater than the probable advantages, but that the European market, our principal one, set great store by constancy of grade qualities. That is, millers are greatly put out by a shipment which falls notably below

the average quality. That is a continuing requirement we must meet, and carlots of 10 per cent protein wheat obviously present a problem and a hazard. Moreover, there has arisen since then a new factor in the form of unparalleled wheat surpluses in the chief producing countries, with consequently enhanced competition for markets.

Altogether it seems to members of the Associate Committee on Grain Research, a national body of which I have the honour to be chairman, that the time has come to reconsider the permissibility of allowing low protein wheat to go into the export grades. Advances in cereal chemistry bid fair to overcome the technical difficulties which faced us at the time of the earlier inquiry, and the comprehensive statistics now available on yields and protein contents in various districts enable us to assess the probable quantitative effect of any restrictions imposed. The exclusion from the main export grades, No. 1 and No. 2 Northern, of wheat falling below 12.5 per cent protein seems to be feasible and desirable. We must expect local opposition in the regions immediately affected, but it is hoped that considerations of the greatest good to the greatest number will prevail.

These considerations obviously affect the northern limits of wheat production, in the sense that wheat grown beyond the 12 per cent line on the northern periphery of our wheat protein maps should be intended for local consumption, either as human food or as stock feed, rather than as an article of commerce. In spite of this limitation, the northward extension of this crop can play an important part in supporting local settlement and the development of the mineral and other natural resources of Canada's richly varied northland. Consequently, scientific studies of the factors involved in growing wheat in northern latitudes, of which I have presented a few in this address, seem amply justified.

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EXPLANATION OF PLATES

PLATE I

Wheat plants after twenty-eight days at 65° and 1800^{ft}f.c.

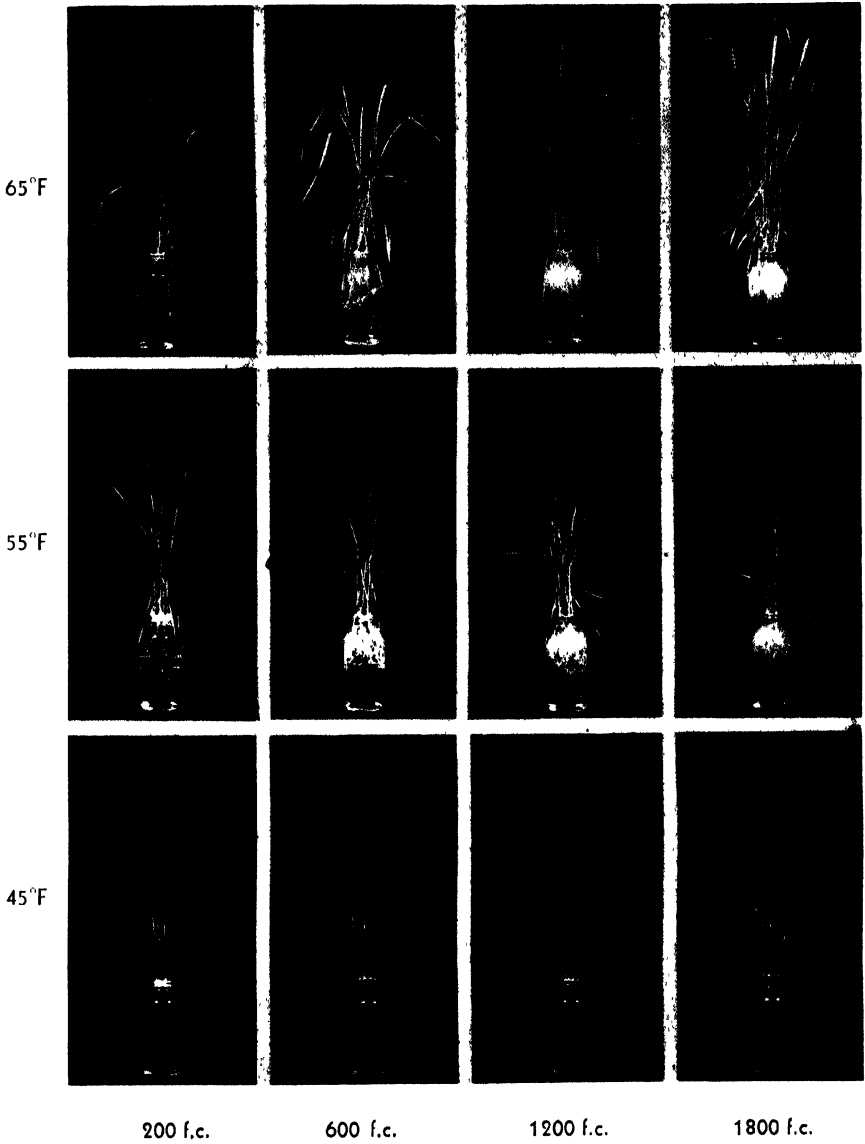
PLATE II

Wheat plants after twenty-eight days.

PLATE I



PLATE II



THE SYMPATHICOTROPIC CELLS IN THE OVARIES OF FŒTUSES AND NEW BORN

A critical study of the interstitial and phæochrome cells as described by

VON WINIWARTER

BY LOUIS BERGER

Presented by L. C. SIMARD, F.R.S.C.

WHEN the sympathicotrophic cells in the ovaries of adult women were established as a new species (Berger, 1923), von Winiwarter objected and considered these cells to be identical with the phæochrome elements he had described in ovaries of fœtuses (1910). Although several inferential arguments could be adduced against such a homologation, a definite refutation could not be made because of the differences existing between the fœtal and adult structures of the ovaries. Inasmuch as von Winiwarter's papers on these cells and on the embryology of the ovary had become almost classics and as his assertion was in disagreement with our own conclusions, we thought it worth while to investigate eventual sympathicotrophic cells also in fœtal ovaries and to select material resembling closely that used by the Belgian author. Thus, complete serial sections of over forty pairs of ovaries, from fœtuses of 20 mm. CR length to the time of birth, were made, the stages corresponding not only to those described by von Winiwarter, but comprising also intermediate aspects.

Inasmuch as eventual sympathicotrophic cells could only be mistaken for either phæochrome or interstitial cells and could hardly be confused with germ or genitaloid cells, we may restrict our description to the former cell species. We shall, however, consider as interstitial or phæochrome, only cells which show distinctive features and resemble either the embryonic testicular interstitial cells or the phæochrome cells in the periaortic or Zuckerkandl bodies of corresponding ages respectively. Contrary to von Winiwarter we will not take account of "young" or "intermediate" aspects, for we became convinced during our studies, that the intermingling of developing, regressing and degenerating cortical and medullary elements and the modifications in the rete blastema led to such complicated aspects, that only fairly differentiated cells could be identified with any reasonable degree of certitude. As comparative standards, we examined many embryonic testes and periaortic paraganglia of corresponding ages.

Interstitial cells could not be found in ovaries of embryos below 100 mm. CR length. Von Winiwarter described "young" interstitial cells in the basal core and between the medullary cords of embryos of from 4 to 9 cm., such cells extending even into the hilus and mesovary towards the latter stage. Close scrutiny of these regions in our own material of corresponding ages did not show any cell resembling even remotely the testicular interstitial cells, which begin to appear as early as at 32 mm. CR length and are fully developed and numerous at 45 mm. when they show very characteristic features. Von Winiwarter's "young" cells, which curiously enough would remain "young" from 40 to 90 mm., either are ordinary, but particularly large and proliferating fibroblasts or are vasculogenic or vegetative (epithelial) cells or correspond to regressing elements of the deep cortical or the medullary cord portions.

Cells closely resembling their testicular counterpart were first seen in the ovaries of an embryo of 110 mm.: they are rather large, with a round, finely structured, generally eccentric nucleus and with an acidophilic densely granulated protoplasm; the latter shows often a clear, vacuolated border zone and contains many peculiar round, slightly elongated or short rod-like, either sharp-lined or indistinct, coarse condensations. The cells may be round or elongated; their outlines are often blurred. They rest predominantly near or along the deep border of the genital layer, in the connective or vascular septa; isolated interstitial cells were only exceptionally found higher up in the genital layer. Their predominant situation is, therefore, in the region where the deepest cortical cord cells mingle with the remnants of the medullary cords. The cells are always scarce, most sections containing only one or two small groups or strands of from two to ten cells.

The number and features of these cells remain about the same up to 180 mm. None were present in the hilus or the region of the rete blastema.

In ovaries of from six lunar months up to the time of birth, these isolated interstitial cells have become still scarcer and are often entirely absent. From the sixth month on, however, a few of the deepest lying primitive follicles, contiguous to the medullary core, evolve into atretic follicles with a generally well developed theca interna. The cells of the latter are of irregular size and shape, but many show exactly the same nuclear and protoplasmic features as the interstitial cells of earlier stages. We, therefore, concur on this point with von Winiwarter, that the interstitial cells from the sixth month on become almost exclusively located in the theca interna of the deep atretic follicles.

As to the phæochrome cells, von Winiwarter identifies as such all cells in any way connected with nerves. He describes two aspects: (1) cells which are interspersed in strands or groups between the nerve fibrils and make the nerves swollen or fusiform, and (2) larger nodules, which are annexed to nerves and may be visible with a magnifying glass. The former are found in nerves of the mesovary and of the very near portion of the large ligament and in nerves of the hilus, sometimes close to the genital layer; the latter are located in the more distant parts of the large ligament and only exceptionally in the region adjoining the mesovary. Now, von Winiwarter states explicitly that the intranervous cells show some coarse, granular or short rod-like protoplasmic inclusions and that the latter are lacking in the cells of the larger nodules, but nevertheless identifies both as phæochrome elements, although his tissues had been fixed in Flemming's solution, which is very unsuitable for any objective appreciation of chromaffinity (Lison).

These two aspects were also found in our own material, but showed several discrepancies from von Winiwarter's observations: the nodular structures were found as early as at 45 mm. between mesonephric elements, when no intranervous cells were present and before interstitial cells appeared, and showed the very same aspect as the periaortic phæochrome bodies of the same age; in later stages they are found only in some glands, were located further away in the large ligament and showed a distinct chromaffinity when fixed in appropriate chrome solutions. The intranervous cells, on the contrary, showed only after the fifth month and increased from then on, were present in all glands, situated in the hilus and the mesovary and the very near region of the large ligament and never showed any trace of chromaffinity; many of these cells contained irregular, ill-defined or sharp, coarsely granular or occasionally short rod-like cytoplasmic condensations. Now we have shown above, that very similar inclusions are a frequent feature in most isolated interstitial and theca interna cells, as they are in the Leydig cells of foetal testes, but they could never be found in any cell of the larger nodules, or in any periaortic phæochrome body. The other nuclear and protoplasmic characters of the intranervous cells were also the same as those of interstitial or theca interna cells and different from the washed-out aspect of phæochrome elements. The close resemblance between the intranervous cells and interstitial and theca interna cells on the one hand, and between the larger nodules and the periaortic phæochrome bodies on the other hand, and the definite differences between both sets of cells are distinctly demonstrated in these figures (lantern slides).

One may, therefore, conclude that von Winiwarter's results must be

revised and corrected. With regard to the interstitial cells, they are almost confined to the deeper part of the genital layer and to occurrence as theca cells around such follicles as may arise, from the sixth month on, at the same level. Before that age their number is always low. The "young," immature and intermediate, aspects described by von Winiwarter could not be definitely ascribed to evolving interstitial cells, the less so as many more adult cells should have been found, if von Winiwarter's immature forms were potential interstitial cells. As to von Winiwarter's phæochrome cells, only those forming juxtaneuronal nodules were ascertained to be such. The intraneuronal cells, responsible for the bulging of the nerve bundles, are not phæochrome, but are morphologically identical with the interstitial cells and with the theca cells of the deep atretic follicles. It appears, that von Winiwarter mistook the intraneuronal cells for phæochrome elements because of their nervous connexions, for he wrote in one of his papers: "If these elements were not situated in the interior of nerves and surrounded by nerve fibers, one might mistake them for interstitial cells." There seems, thus to be little doubt left, that he fell victim to the *petitio principii*, that only phæochrome cells showed intimate relations with nerves and that inversely all cells with close nervous connexions must be phæochrome.

Our investigations prove, therefore, that cells identical with the interstitial or theca cells of the foetal ovary may appear in and around the nerves of the hilus, the mesovary, and the neighbouring part of the large ligament. These cells are the foetal counterpart of the cells having identical nervous relations in the adult ovary, where we have called them "sympathicotropic cells." However, whereas the sympathicotropic cells of the adult ovary are surprisingly identical with the Leydig cells of the adult testis and are different from any other ovarian cell, the foetal sympathicotropic cells appear closely like the interstitial and theca cells of the foetal ovary. No direct continuity could be found, however, between the latter and the cells in the nerves: as in adult ovaries the foetal sympathicotropic cells seem to arise where they are found. These discrepancies in likenesses of foetal and adult sympathicotropic cells to other cells in the gonads raise questions not only of great embryological and biological interest, but also of much eventual pathological importance.

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SOME WAR-TIME FOOD AND SUPPLY PROBLEMS

By W. H. COOK, F.R.S.C.

INTRODUCTION

THE urgency of war directs activity along the line of applications and development rather than toward fundamental contributions to knowledge. Biological research generally and the activities of the Division of Applied Biology in particular, have dealt largely with the "defensive" type of problem, lacking the spectacular element implicit in developments of an "offensive" character. This paper deals with some of the contributions of the staff to the prosaic problems of total war: a task usually lacking either the stimulating interest of fundamental work or the hidden feeling that success would yield a secret weapon.

The defensive type of problem must be anticipated if useful progress is to be made before the actual need arises. Such work inevitably contains its complement of negative results. Some problems were not foreseen, and others that were anticipated did not arise. Results applied in practice represent the real contribution, but a precise evaluation is impossible.

The Division's war investigations have varied considerably as to subject and scope. Obviously, they cannot be discussed in detail. It seems best to review the major problems in general terms, indicating how the various projects came to be undertaken, the results obtained, and their relation and value to the war effort.

In retrospect the war period can be divided into seven phases as far as its effect on the Divisional activities is concerned. The outlook was different during each period, resulting in a somewhat different type of problem having the greatest urgency. Each of these phases marks a period of initiation rather than termination, since the work undertaken at one stage was seldom complete before the next phase brought a new class of problem.

The first was a period of expectancy, extending from the outbreak of hostilities to the time of Dunkerque. The second, extending from Dunkerque to early 1941, was a period of anxiety. Food storage and transport problems attained considerable importance during the next phase, one of shipping shortages, which extended through 1941 and 1942. The events that followed Pearl Harbour introduced the fourth period, one of strategic shortages of certain raw materials. The year 1943 brought concern as to world food supplies and during this fifth

phase food investigations assumed a new importance. The problem of fighting nature as well as the enemy introduced the sixth phase late in 1943 when military activities in the South-West Pacific area had shown that supplies would have to be climate-proofed. We are now entering the seventh phase involving the assessment of certain war investigations in relation to the Pacific campaign and the national economy of the future.

PERIOD OF EXPECTANCY

At the outbreak of war the Division of Applied Biology had a total staff of thirty-seven. Less than half of this number were trained scientists. The largest individual group was engaged in the general field of plant science, including plant breeding, plant hormones, plant growth, and related studies. The next largest group was engaged in food investigations and, in particular, bacon curing and transport. A still smaller group was working in the field of fermentations, primarily on lignin decomposition, while other individuals were engaged in statistics and other specialized fields.

With the outbreak of the war none of these activities seemed to have a direct bearing on the war effort. The entire staff were anxious to serve in the most useful capacity, and expected to receive instructions as to what they should do either scientifically or otherwise. With this in mind, their first effort was to wind up in some form the peace-time problems on which they had been engaged.

It has since become evident that this feeling of expectancy existed among many biological groups throughout the Commonwealth, and none received the expected instruction. In retrospect, the expectancy concept was fundamentally wrong. Research programmes, even in an applied field, are seldom if ever undertaken on specific instructions. The hard-pressed administrator may be the first to hear of really worthwhile problems, but he frequently fails to recognize them as capable of scientific study, and even if he does, it still requires a technically trained man to reduce them to the specific terms necessary for laboratory investigation.

Lacking specific instructions, investigators towards the close of 1939 felt that they could anticipate certain food problems.

Preservatives for Bacon

Food transport problems had arisen in the last war when bacon had to be shipped in unrefrigerated space. Borax was used then to avoid spoilage, but the eating quality of the resulting product left much to

be desired. Were such problems likely to recur? It seemed probable that they would. Over one hundred preservative treatments were therefore tested extensively and part of the work has already been published (White, Gibbons and Thistle, 1945; White, Thistle and Steeves, 1945). Earlier studies had indicated that smoking prior to shipment might contribute substantially to the keeping quality and this possibility was investigated in some detail (Pearce and Woodcock, 1945; White, 1944; White, Gibbons, Woodcock and Cook, 1942; White, Woodcock and Gibbons, 1944).

Briefly, the more effective preservatives improved the keeping quality to the extent of approximately doubling the storage life at a given temperature. Smoking under the best conditions gave similar results, retarding bacterial development and the onset of rancidity but enhancing mould growth. While some of these treatments, and smoking in particular, offer distinct possibilities for extending the storage life, none was assessed as adequate to permit shipments in unrefrigerated space. At the present time, the subject has been reopened and it seems likely that smoked meats will be forwarded for relief feeding in Europe.

Canning Investigations

When Britain cancelled her imports of dressed poultry, Canada was threatened with a surplus, demanding valuable cold storage space for its preservation. Canning was an obvious relief measure, and at the request of the Department of Agriculture work on canned poultry was undertaken early in 1940. The investigation was primarily a routine study of initial product quality, processing procedures and methods of control. Substantial assistance was also given to commercial operators. Some of the work has been published (Reedman, 1943; Reedman and Buckby, 1943; Reedman, Buckby and Legault, 1942), but the main object was to provide an adequate specification which is now incorporated in the Meat and Canned Foods Act.

As the war progressed, the Allied Forces had need for canned meat in 6-lb. containers that could be held at desert temperatures without deterioration. Prior to that time, most meats packed in containers of this size were subject to spoilage even at ordinary temperatures. Complete sterilization by current methods resulted in an over-cooked, unacceptable product in which a considerable body of liquid separated and was lost as waste. This problem was investigated at the request, and with the co-operation, of Department of Agriculture officers and industrial firms. Suitable processes were worked out and assistance given to commercial firms.

Blood Storage

The early part of the war saw considerable interest in the use of whole blood for transfusions. Because of the facilities available in the food laboratories of the Division for storage of products at any desired temperature, the Medical Committee requested that some work be undertaken on blood storage. Supplies of human blood were donated by the staff and collected with the assistance of medical personnel from local hospitals.

The object of the investigation was to find the storage conditions that would cause the least haemolysis. The results (Thistle, Gibbons, Cook and Stewart, 1941) showed that: adding glucose markedly retarded haemolysis; the best storage temperatures lay between 2.5 and 6°C.; and also that excluding air and keeping the blood in slow motion reduced haemolysis. Even under the best conditions, however, the blood could only be stored for about four weeks.

The storage life of whole blood might reasonably be extended if it could be frozen without destroying the erythrocytes. Some studies were made on the freezing of sheep cells using protecting agents and extremely rapid freezing rates (Woodcock, Thistle, Cook and Gibbons, 1941). Methods were developed that resulted in the survival of a substantial number of cells, but this part of the programme had little of practical value.

All of the above studies were terminated when plasma came into general use. Subsequent assistance was given, however, on development of containers for shipping whole blood from centres of collection to the laboratories engaged in serum separation and drying.

PERIOD OF ANXIETY

The problems just described were engaging the attention of only a fraction of the original staff at the time of Dunkerque. Following this there was an immediate appeal to be transferred to problems likely to be of direct value in the war effort. A few had already left to join the armed forces and war industry. There was now a more general exodus.

By this time it had become evident that if problems of direct interest to the war effort were to be undertaken they would have to be conceived by the staff themselves. Some of the personnel started to acquaint themselves with the problems and techniques of chemical warfare. Most of the problems selected were of an elementary sort, designed largely for training purposes, although some were original. One small study arising out of this work, but having no wartime significance, has been published (Rosser, 1941).

About a year later the Directorate of Chemical Warfare and Smokes was established under the National Defence Department. The Division's laboratory space, a senior investigator and the personnel who had been trained in the interim formed the initial physiological section of this establishment.

Statistical Section

During this period and subsequently the statistical section found a place for its special training and experience. The planning of physiological tests in connection with the chemical warfare work was one of the first problems. Additional studies were undertaken on the fitting of gas masks to Canadian army personnel, involving the taking and analysis of over fourteen thousand facial measurements.

A statistical examination was made on the strength of spot weld in aircraft in order to provide adequate material for drafting specifications. Still later, work was undertaken on the nutritional status of troops in local depots.

Finally, in 1942, Dr. J. W. Hopkins, who headed the statistical section, joined the Operational Research Group of the R.C.A.F. and is the only officer originally appointed to this Group who has served in England with No. 6 Bomber Command.

Army Rations and the Vitamin Content of Foods

During this period the British Ministry of Food was considering the addition of thiamin to imported flours. This brought up a number of routine questions as to how the small amount of vitamin could be added uniformly, simple methods of checking the vitamin content in commercial practice, and standardization of methods between British and Canadian laboratories. This resulted in the development of equipment and methods (Reedman and Young, 1943; Woodcock, 1941) for determining thiamin.

In 1941, the National Defence Department appointed the Standing Committee on Nutrition to advise the Minister on matters pertaining to army rations. Vitamin C in the army ration appeared to be rather low during the winter months and the Committee recommended changes to remedy this defect. The Divisional investigators undertook to assess the position by measuring the intake of vitamin C in two military depots. It was found (Hopkins, Marshall and Creasy, 1944) that the intake varied substantially between individuals, but the average intake of 22 mg. per day was far below the recommended daily intake of 75 mg. In spite of this, there were no gross symptoms

of vitamin C deficiency. This problem could not be resolved without an accurate means of bioassay. Since facilities for animal feeding experiments were lacking, support was given for this work to be undertaken at MacDonald College (Crampton and Farmer).

The need for an operational ration in the Canadian army resulted in the Canadian Mess Tin Ration. Following the initial field trials these rations were examined in the laboratory. This examination showed that some of the components would have to be altered to increase their stability and that the packaging of most of the items would have to be improved. This led to the preparation of specifications for the biscuit shortenings, then for the biscuits themselves, and finally for all items including packaging. While a number of these specifications were based on existing knowledge, others had to be confirmed by laboratory investigation (Grant, Marshall and White, 1945; Pearce and Marshall, 1945). The work on biscuits alone involved nearly two tons of experimental material.

Another problem was concerned with finding a suitable carrier for vitamin C in this dry ration. The storage qualities of dehydrated citrus fruit juices, and of synthetic vitamins added to different types of candy, chewing gum and jam were investigated (Marshall, Hopkins and Young, 1944). Candies were chosen as the carrier for this vitamin. Similar work was done on beverage powders in relation to their palatability and suitability as carriers for the fat-soluble vitamins. Still later, the Associate Committee on Army Medical Research requested information on vitamin C content of sprouted seeds; dehydration of materials used for supplementary feeding of convalescents; and studies on fats. Work on these problems was initiated in the Division with assistance from other institutions.

PERIOD OF SHIPPING SHORTAGES

The shipping shortage resulted from: first, the use of convoys which slowed marine traffic; second, the loss of European sources of food supply which meant longer voyages; and finally, losses of shipping through enemy action. Perishable food products are required in large volume, and demand refrigerated shipping space. The situation became serious early in 1941 and again in 1942 following Japan's entry into the war. Many problems were referred to the food investigators during this period.

Bacon

Loss of European supply sources increased the demand for Canadian bacon. Pre-war curing practices in Canada had contemplated a

ten-day voyage. The use of convoys extended this period to twenty days or more, and some lots of bacon were discharged in poor condition. This brought a request for harder cures (higher salt content) and the specific suggestion that this be obtained by longer curing periods. Our pre-war work had shown that the curing periods used in different Canadian plants varied over 100 per cent and that the rate of up-take of salt was negligible after the first few days. Increased curing periods would therefore have been of little value and would have demanded additional curing facilities at a time when labour and materials were scarce and the bacon trade was expanding rapidly.

The Divisional investigators recommended standard curing practices in all plants by methods that would increase the salt content while decreasing the average time required for curing. With one minor exception these recommendations were accepted by industry and the Canadian and British officials concerned. While this war-time cure is somewhat too salty for the British palate, the reports received on this product under war-time conditions have been highly satisfactory.

Emergency Refrigeration

These changes still contemplated the shipment of bacon in refrigerated space at temperatures near the freezing point. By April, 1941, the critical shortage of refrigerated shipping space resulted in the formation of a special committee in Britain to consider means for maintaining perishable food imports. A direct cable was received from the Food Investigations Board requesting all possible assistance in the solution of this urgent problem, and similar requests were received by other bodies. The Meat Board convened a meeting and most of the discussion centred around the use of borax or other preservatives. Since our own experience had indicated that such practices would be neither effective nor desirable, it was suggested that ordinary cargo holds be converted to refrigerated space by the installation of emergency refrigerating equipment. Authority was granted to examine the possibilities with the shipping companies, supply firms, and the Ministries of Food and War Transport. This survey revealed scepticism on the part of nearly every group, but it was accompanied by full co-operation in the best sense of the word.

The proposed scheme required vessels having the necessary water and electric power supply, and the use of lower holds to minimize heat transfer. These requests were granted on the understanding that installation of the equipment would not delay the vessel; and that the holds selected must not be obstructed by air trunks or other permanent gear.

Prefabricated unit equipment went a long way toward meeting the first demand but the application of insulation in the ordinary sense was impossible. With nothing but the steel plate of the hull and the cargo battens between the sea water and cargo, the only resistance to heat flow lay in the gas film on the plate. Actual measurements of the film coefficient on vertical plates under these conditions were lacking and in consequence the refrigerating load had to be calculated on rather shaky theory. The prefabricated equipment demanded a cold air system, and a few problems arose as to how this could be done without the use of ducts, but as details have been described elsewhere (Cook and Steeves, 1942), they need not be given here.

The vessel named for the initial trials was the *Vancouver Island*, previously the *Weser*, captured by the *Prince Robert*. With the co-operation of the Department of Agriculture, and shipping and industrial firms, the first installation was made without delaying the vessel. The results are summarized by a cabled message: "Cargo discharged in satisfactory condition."

Improvements, including the erection of blanket insulation, were made on the next voyage. Automatic defrosting of the coils was provided on the third trip, but on this voyage the ship went the way of all guinea-pigs. Fortunately, the progeny of the original effort remained. A similar installation had been made on another vessel in New York. At the request of British investigators still another vessel was provided with additional refrigerated cargo space by extending the existing refrigeration system to cool additional ordinary cargo space. This had the advantage of simplifying the installation and minimizing the need for new refrigerating equipment, then in short supply. Subsequently a substantial amount of shipping space was converted in this way.

Some of the later applications are of interest. A fully insulated and refrigerated vessel, with fourteen holds, which had lost her permanent refrigerating equipment was fitted with emergency units. A smaller vessel was fitted to carry frozen meats to service personnel in bases along the north-east coast of Canada. Last year the rapid advance of the United States forces in the war against Japan raised problems in maintaining bases for perishable food supplies. Refrigerated warehouses were soon left far behind the scene of operations. An officer of the United States Quartermaster Corps asked us to provide a design for a refrigerated barge that might be towed from island to island as the fighting front advanced. The first of these barges embodying a number of the suggestions put forward went into action not long ago.

Eggs

Loss of European sources of supply brought a demand for Canada to supply eggs for Britain. Eggs-in-shell have a morale, as well as a nutritional, value, although they require considerable shipping space. Canada had exported a negligible quantity of shell eggs to Britain prior to the war and shipment in any form, far less in unrefrigerated shipping space, had not been anticipated. During the warmer parts of the year, slow convoy transport in unventilated and unrefrigerated holds resulted in losses at a prohibitive level by ordinary standards. An immediate study of preservative treatments was undertaken, but as the problem had not been anticipated the experiments had to parallel commercial shipments. While useful information was obtained (Gibbons, Fulton and Hopkins, 1942; Reedman and Hopkins, 1942; Rosser, 1942; Rosser, White, Woodcock and Fletcher, 1942), no fully effective solution could be worked out on short notice. As a result shell egg shipments were cancelled early in 1942 and experimental work was reduced to a minimum. These shipments have now been resumed and a combination of the more effective preservatives and practices has resulted in the product reaching Britain in far better condition.

Dried Eggs

When successful shipment of shell eggs in unrefrigerated space looked hopeless it was felt that dried eggs might offer a solution to the problem. Liaison was established with D.S.I.R. investigators in Britain, who were also working on this possibility. The correspondence can be summarized in two questions. Canada: Why not dried eggs? Britain: Can you eat them? While dried egg was an article of commerce, most of it had been used for cooking or baking purposes. What Britain wanted was a high quality product that could be eaten as an egg dish as an alternative to shell eggs.

Samples of dried egg of fresh and ancient vintage were obtained from Canadian and American firms. Existing chemical tests indicated that they were satisfactory but most of the staff agreed that they were unsuitable when cooked as scrambled egg. Samples sent to British investigators confirmed this opinion. Before the process of egg drying could be studied and effective means taken for improving quality, an objective yardstick of quality was essential. About this time the British investigators had worked out a subjective flavour score. While this was a useful tool, it scarcely seemed adequate for experimental or grading purposes.

The search for a yardstick culminated in the use of a fluorescence test (Pearce, 1943; Pearce, 1944; Pearce and Thistle, 1942; Pearce,

Thistle and Reid, 1943; Thistle, Pearce and Gibbons, 1943), which had been worked out in principle about the time shell egg shipments were cancelled. Following this the facilities for drying eggs had to be expanded several times, bringing the staff many problems on drier design and operation (Woodcock and Tessier, 1943). This period provided an excellent opportunity for assessing the new tests, determining the drying conditions necessary for producing a high quality product, and preparing adequate specifications. The fluorescence test was included in these specifications, and was subsequently adopted by the United States Quartermaster Corps, and in modified form by the British Ministry of Food.

The majority of the results (Pearce, Woodcock and Gibbons, 1944; Thistle, Reid and Gibbons, 1943; Thistle, White, Fletcher and Pearce, 1945; Thistle, White, Reid and Woodcock, 1944; White and Grant, 1943; *ibid.*, 1944; White and Thistle, 1943; White, Thistle and Reid, 1943; Woodcock and Reid, 1943) have been published. They showed that dried-egg powder was still a perishable commodity and temperature and moisture content at all stages was most important in determining the quality of the final dried product. As this information became available, specific instructions were drafted covering equipment, processing methods, storage and transport conditions, and grade standards. Subsequently Canada gained a reputation for supplying the best dried egg available in Britain.

At one time British personnel were concerned about the possibility of *Salmonella* and other toxic organisms being present in the powder. Since fowl generally are a source of many types of *Salmonella*, and since the egg liquid does not reach a pasteurizing temperature at any stage in the process, it was felt that there might be some hazard in its general use as an egg dish. Relatively extensive studies were made on the *Salmonella* (Gibbons and Moore, 1944; Gibbons, Moore and Fulton, 1944) and bacterial (Gibbons and Fulton, 1943) content generally of dried-egg powder. While *Salmonella* were occasionally found in Canadian powders, the level was generally low and it was found that a substantial number of the organisms did not survive in the dried product.

PERIOD OF STRATEGIC SHORTAGES

The events that followed Pearl Harbour brought strategic shortages in certain raw materials. In particular the reduced supplies of rubber, agar and tin brought a new class of problem to the Divisional investigators.

Rubber from Canadian-grown Plants

When we were faced with an acute rubber shortage, interest was aroused in the possibility of obtaining rubber from Canadian-grown plants. Russia had produced commercial quantities of rubber from dandelion (*Taraxacum kok-saghyz* Rodin). Milkweed (*Asclepias syriaca* L.) was known to contain rubber and was plentiful in Eastern Canada. Other plants had possibilities as sources of rubber. A programme involving all phases from problems of production to assessment of rubber quality could only be carried through by co-operative effort. The work involved the Department of Agriculture, and the Universities of Toronto, Queen's and McGill in addition to the Divisions of Applied Biology and Chemistry of the National Research Laboratories.

The Division's part in the programme was to study methods of extraction since solvent procedures were unlikely to prove satisfactory for processing large quantities of material of low rubber content. Alkali digestion of the dried material followed by pebble milling alone or in combination with froth flotation was found to extract the resin-rubber gum from both milkweed and kok-saghyz (Grace, Watson and Klassen, 1944; Klassen and Grace, 1945; Krotkov, 1945; Paul, Blakers and Watson, 1943; Turrall, Klassen and Smedley, 1943; Watson and Grace, 1944). Milkweed rubber was found to be of poor quality but at one stage it appeared that it might be useful for blending with synthetic rubber. Kok-saghyz rubber was superior in quality being almost the equivalent of that from Hevea. High costs and difficulties in crop production, combined with the rapid advance and success of the synthetic rubber programme, led to this project being discontinued.

2, 3 Butanediol

Early in 1942, it was evident that synthetic rubber of the Buna S type would have to supply the majority of the rubber requirements of the United Nations during the war period. Butadiene, made from either petroleum or alcohol, was required for its manufacture. Alcohol supplies would have to be produced from grains rather than molasses owing to the shortage of shipping. Cereal grains were available in surplus proportions and, while their fermentation to alcohol was well understood, some assistance was given in the conversion of plants from potable to industrial alcohol production.

It was also felt that other possibilities should be investigated. Since butadiene is a 4-carbon compound it was felt that production of a 4-carbon compound by fermentation might be of some value. The

fermentation group proposed the production of 2, 3 butanediol, one of the few 4-carbon compounds produced by fermentation. Some months later, it was found that similar investigations were under way in the United States. Divisional studies were then co-ordinated with the American programme, and the effort intensified with the co-operation of the Dominion Department of Agriculture and the University of Alberta.

The initial object of the work was to put butanediol production on the same basis as alcohol production with respect to yield of products and time of fermentation. Laboratory work was undertaken with *Aerobacter aerogenes*, which yields a mixture of the *meso*- and *d*-forms of the diol. This fermentation requires saccharification of the starch as in yeast fermentation, a high degree of sterility with respect to other organisms, and aeration during fermentation. Since malting and aeration did not appear to be compatible with the maintenance of sterility, further studies were deferred in favour of studies on another organism, *Aerobacillus polymyxa*. This bacterium produced diastatic enzymes, and did not require aeration, but about a third of the product obtained was alcohol. The butanediol fraction, however, was the *l*-isomer which has more useful physical properties than the other isomers. Toward the end of the year the initial object had been achieved in the laboratory.

In order to carry the work a stage further, the Division made application for a pilot plant and this was approved by all authorities, including the War Production Board. The pilot plant was designed and erection completed early in 1944. Since that time the laboratory processes with respect to yield and time have been confirmed.

The critical period of synthetic rubber production has now passed; secrecy restrictions have been cancelled and several papers have been published (Adams and Stanier, 1945; Ledingham, Adams and Stanier, 1945; Neish, 1945; Rose and King, 1945; Stanier, 1943; Stanier and Adams, 1944; Stanier, Adams and Ledingham, 1945; Stanier and Fratkin, 1944). There is still considerable interest in this product for direct use as an antifreeze and for conversion to other chemicals, one of which appears to be superior to those in current use for mould proofing fabrics for tropical climates.

Irish Moss (Chondrus crispus Lyngb.)

Although the most important use for agar is in the bacteriological laboratory, a far greater volume of this jelling substance is used in the food industry. When Japanese sources were cut off, the available supplies of agar were brought under control and at the same time we were

asked to investigate possible gel substitutes for use in the food industry. Irish moss appeared to be one of the best materials since it is a Canadian product, harvested in substantial quantities since the war, and in current use in food and pharmaceutical industries as a stabilizer or thickening agent.

Aqueous extracts of Irish moss, while extremely viscous at 2 to 3 per cent concentration, seldom have the properties of a true jelly. As a stabilizing agent they are not required in concentrations in excess of a fraction of a per cent, and at such dilutions the colour or flavour of the extract is not detectable. The problem was, therefore, one of obtaining an extract with the desired jelling properties and sufficiently free from colour and flavour to permit its use in higher concentrations. Earlier findings indicating that the addition of potassium chloride resulted in a satisfactory jelly, were confirmed (Reedman and Buckby, 1943). Treatment with carbon black reduced the colour and flavour to a level that permitted its use in 2 per cent concentrations. Special techniques had to be developed to permit the subsequent removal of the carbon black from the viscous solution. Since the solution containing potassium chloride set to a jelly on cooling, a method of concentration based on freezing rather than drying by evaporation was developed. Tests on food products both in the laboratory (Fulton and Metcalfe, 1945) and in commercial plants have proved satisfactory. A commercial firm is now erecting a pilot plant in Canada based on this process.

Packaging

The shortage of tin plate demanded the use of alternative methods of packing wherever possible. Dehydrated products had to be packaged in highly water-vapour resistant materials that would stand up to the ordinary handling operations incidental to filling and subsequent transport. Cartons dipped in wax or asphalt could not be used for goods shipped to Britain, since she required pure kraft to strengthen re-pulped stocks, and dipped cartons would have made satisfactory re-pulping impossible.

These requirements were met by using a bag-in-carton package. The bag was made from specially coated heat-sealing cellophane, protected by a pure kraft carton. This package (Woodcock, Chapman and Pearce, 1945), designed by the Divisional investigators, has carried all of the dried egg shipped to Britain for nearly three years. This is something on the order of fifteen thousand tons, in two types of container holding 5 oz. and 14 lbs. respectively.

Certain products, such as whole milk, must be protected from oxygen as well as water-vapour, demanding vacuum or gas packing in metal containers. Laboratory investigations (Woodcock, 1945) showed that laminated cellophane transmitted carbon dioxide about twenty-six times as rapidly as oxygen. Hence, the outward diffusion of carbon dioxide produced a modified form of vacuum pack. Defective packages could subsequently be detected readily since they remained soft, while sound packages became relatively hard from compression at atmospheric pressure. Laboratory and commercial tests have been made and the results are promising.

From these early developments the Division subsequently became responsible for much of the test and development work required by the Canadian Packaging Committee. Present activities include packaging not only of foodstuffs, but a wide range of instruments, spare parts, and practically all service items for tropical, temperate and Arctic climates.

WORLD FOOD SUPPLIES

Shortages of other food and supply items became evident during 1942 and 1943. While local shortages could be attributed to lack of shipping, it became evident that there was a real shortage of world food supplies. This consideration formed the background for the Hot Springs Conference where plans were formulated for the future. The immediate situation was even more serious since the close of the war would bring the problem of feeding the populations of the liberated nations. This need demanded additional foods that could be shipped and stock-piled for immediate use where required. The combined food boards undertook this problem. In 1943 they sent a mission to South America to see if additional foods, particularly animal proteins, could be procured. The author had the privilege of being a member of the British team on this mission. My part was concerned with the concentration of the material and reduction of its perishability so that it could be stock-piled.

This world food position was naturally of direct interest to Canada. Dehydration was one of the best methods for concentrating and reducing the perishability of animal products. During this period studies on egg dehydration were intensified; early work of a preliminary nature on the dehydration of pork (Pearce, 1943; Pearce, 1945) was completed and investigation of dried milk (Pearce, 1945) was undertaken.

Starch and Sugars

Canada's domestic supplies of starch and sugar became exceedingly limited during this period. Previously most of the starch used on the North American continent had been made from corn, and the equipment and methods employed were not suitable for extracting starch from other cereals. Corn available to Canada was reduced first as a result of the shipping situation and later by a supply shortage, since alcohol production for rubber demanded large amounts of corn. These supply limitations occurred at a time when greatly increased quantities of starch were required directly by war industry, and for the production of syrups and glucose to augment the short supply of sugar.

Wheat starch could fill these requirements if a suitable commercial procedure could be developed for its separation from flour or wheat. Early investigations on starch-gluten separations were therefore completed during this period. While they were not particularly promising as a commercial procedure (Adams, Ledingham and Grace, 1945; Grace, 1944; Grace and Clendenning, 1945), they provided a background of knowledge that led to a satisfactory continuous process, applicable to flour or finely ground wheat. Briefly, the procedure consists of continually mixing the flour and water to a dough and aging and slurring the resulting dough in an excess of water. This slurry is then pumped to a rotating screen which separates the starch and gluten fractions. The process yields over 90 per cent of the starch present and it contains less than 0.1 per cent nitrogen.

Starch of this purity is satisfactory for the production of glucose and syrups and the hydrolysis of wheat starch is now under investigation. Other possible sources of sugars have been examined (Johnson, 1944).

Edible Fats and Oils

Shortages in domestic supplies of edible fats and oils occurred during this period, and requests for information and investigation of certain subjects were received from the Fats and Oils Administrator during 1942. With the co-operation of the Associate Committee on Grain Research, the situation was surveyed with respect to availability of commercial oil processing equipment and the best method of attacking the problem experimentally. Much information had to be obtained on the keeping qualities of oil-bearing seeds (Larmour, Sallans and Craig, 1944; Sallans, 1944; Sallans and Sinclair, 1944; Sallans, Sinclair and Larmour, 1944).

Linseed oil and lard were the only fatty materials produced in Canada in substantial quantities. New crops yielding edible oils, such as sunflowers, might be expanded but this would require time. Linseed oil had not been considered an edible oil, and lard had certain shortcomings, such as instability, that limited its usefulness.

Investigations on the conversion of linseed oil to an edible shortening were referred to the Ontario Research Foundation (Lemon, 1944) with assistance from Divisional investigators (Lemon, Lips and White, 1945). The work, which is still under way, has not been wholly successful although in subsequent emergencies small amounts of linseed oil have been included in certain shortenings. Drying oils such as linseed revert to their original painty odour and flavour even after hydrogenation. It has been shown that a hydrogenation product of the glycerides of linolenic acid is responsible for this property.

Examination of new oil-bearing crops was carried on jointly by the Associate Committee on Grain Research, the Department of Agriculture, and in the Division, with the assistance of commercial firms. This work involved establishing grade standards for the new seed (Sallans, Berenbom and Larmour, 1945), developing suitable crushing and processing practices both in the laboratory and commercial plants, and finally assessing the quality of the finished product.

The Division accepted the responsibility for investigations on lard. The relative importance of other parts of the general programme has delayed the initiation of this work which is now under way. A good deal of technical advice and assistance has been given in connection with processing procedures, antioxidants, and specifications to meet different requirements.

CLIMATE PROOFING

The war against Japan is also a war against nature. So far most of the military activities have taken place in tropical regions where temperatures of 90°F. and relative humidities of 90 per cent are quite common. Facilities for the proper transport or storage of supplies are exceedingly limited. Food, fabrics, cordage, wood, packages, metal parts and supplies of all kinds are subject to rapid corrosion or attack by moulds, rots or insects.

Tests and small-scale investigations had been conducted on rot resistance, fungus proofing, and fungicides for several years (Fulton, Gibbons and Moore, 1944). Early in 1944 the studies on this subject reached the proportion of a major project and have increased in importance ever since. Essentially the work is one of routine testing, but a research element was introduced by the fact that no prescribed test-

ing procedure was available. Each piece of equipment presents a new testing problem to determine its vulnerability and subsequently to develop proofing methods or packaging procedures which will provide the necessary protection. Some remarkable types and rates of deterioration have been observed but little can be said about this work as yet.

Some work has also been necessary on the effect of Arctic conditions on the deterioration of equipment. To extend these studies the Division has designed and is now erecting a -70°F. test room using a fully automatic cascade refrigerating system based on two modern refrigerants, dicloro - difluoro - methane and monochlor - difluoro - methane.

END OF WAR IN EUROPE

The war has now entered stage two—not the post-war period. Investigational work must now be assessed in terms of its value in the war against Japan and the national economy of the future. Foodstuffs and many other supplies will remain scarce. In consequence food processing, storage and transport investigations and also climate proofing are likely to become even more important during stage two of the war.

Looking beyond the immediate difficulty of feeding devastated Europe there are still many important problems to be solved in connection with perishable foods. War-time bacon cures are known to be too salty for the British palate. Reduction in salt content will increase perishability. This will demand the construction of refrigerated docks at our ports and improved refrigerated shipping facilities. Dehydration of foodstuffs will likely be less important, but dried eggs and milk in particular will remain if processing methods are improved and quality maintained. Substantial progress has already been made in creating a post-war market for dried eggs.

The work on fermentation should prove as valuable in peace as in war. Expansion rather than contraction of projects dealing with utilization of agricultural wastes and surpluses should be the rule as the war draws to a close.

The applied problems undertaken during the war have opened up a vast number of fundamental problems. The nature of the fluorescing substance found in many foodstuffs, the nutrition of organisms producing useful products by fermentation, the nature of antibiotics, to mention only a few. A progressive national economy cannot afford unemployment or misemployment of scientific staff in our time.

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SOME FACTORS INVOLVED IN THE NATURAL
PRODUCTION OF PACIFIC SALMON

By R. E. FOERSTER, F.R.S.C.

THE proper management of the salmon fisheries of the Canadian Pacific coast in order to continue commercial exploitation at a high level is a matter of prime importance. Of recent years declines in the packs of the various species in certain coastal areas have led to concern as to the future maintenance of the fisheries and have resulted in the initiation on the part of the Fisheries Research Board of Canada of comprehensive investigations. It first becomes necessary to determine whether the declines have actually occurred and if so, are they of a cyclic nature and uncontrollable or can they be attributed to preventable circumstances? If the latter, can the limiting factors or conditions be revealed and corrected?

When a salmon fishery begins to show decline the factors popularly held responsible are either over-fishing, thus effectively limiting the seedling and subsequent production of new stocks of fish, or decrease in the productive capacities of the spawning or nursery areas, by reason of floods, freshets, droughts, obstructions, excessive predation, etc. A consideration of these two possible factors and their bearing upon alleged decline in abundance of the five species of Pacific salmon may be timely.

The effect of fishing drain upon reproduction

In the period prior to initiation of commercial fishing, the only strain on the effort of the species to maintain itself at optimum capacity was to overcome those losses occurring from natural mortality during the life-cycle, from the depredations of natural enemies, fishes, birds and mammals, and from whatever Indian fishery existed. In all likelihood these drains were relatively small, hence perpetuation of the stock to the optimum level of the capacity of the spawning and nursery areas was easily achieved. With the females of each species of salmon spawning relatively large numbers of eggs, a very low survival rate only was required to assure the return of two spawners from each pair spawning. Average egg counts vary as follows: pink salmon—1,535 to 1,841; chum salmon—2,760 to 2,943; coho salmon—3,002 to 3,152; sockeye salmon—3,264 to 4,282; spring salmon—3,885 to 8,426 (Foerster and Pritchard, 1936). Abundant leeway was thus provided to meet extraordinary mortality circumstances. No doubt, however, fluctuations in the runs occurred,

due to adverse conditions prevailing on the spawning grounds, freezing, droughts, freshets, etc. and at times the regenerative capacities of the stock may have been taxed.

With the development of the commercial fishery and its increase to the present level of exploitation a distinctly heavy drain was placed upon the regenerative capacities of the stocks. Where the commercial fishery, for example, removed one half the population returning from the sea a 100 per cent increase in the reproductive requirements of the species became necessary to maintain the population under such drain, i.e., four eggs were now required to survive to the adult stage to assure two spawners for each two spawning. The extent to which the regenerative powers of the stock were affected was therefore dependent upon the degree of drain upon the population as exercised by the commercial fishery in reducing the spawning escapement.

The sockeye salmon (*Oncorhynchus nerka*) present perhaps the most important problem because of the fact that the young fish after hatching spend at least one year in fresh water and hence are subject to extra conditions not imposed to the same degree on the other four species.

Investigations to determine the extent of survival in the two main periods of the life-history, fresh-water and marine, have been conducted at Cultus Lake, Fraser River, by the Fisheries Research Board of Canada (Foerster, 1936, 1938a) and on the Karluk River, Alaska, by the United States Fish and Wildlife Service (Barnaby, 1944). These studies have revealed the following:

(a) For Cultus Lake, three tests of natural propagation have given percentage survivals up to the seaward migrating smolt stage, of 1.13, 1.05 and 3.23 per cent of eggs presumed to have been deposited, or an average of 1.80 per cent (Foerster, 1938a, p. 154). From seaward migrant to returning adult stages, i.e., the ocean period, survival was calculated (Foerster, 1936, p. 34) to range from 3.5 per cent to 11.7 per cent, with a most probable value of 9.9 per cent. Total survival, from egg to adult stage, therefore, would approximate 0.2 per cent.

(b) For Karluk River, Alaska, "With a ratio of return to escapement of 2 to 1 the mortality between eggs and seaward migrants would be 99.55 per cent, while with a ratio of return to escapement as high as 4 to 1 the mortality between eggs and seaward migrants would still be over 99 per cent" (Barnaby, 1944, p. 292). Survival during the fresh-water period thus amounts to less than one per cent. Ocean survival is computed at twenty-one per cent, thus providing a total survival from egg to adult stage of from 0.1 to 0.2 per cent.

As Barnaby (1944, p. 247) has pointed out, "In a self-perpetuating salmon population an adequate part of the yearly run must be allowed to escape the fishery and continue uninterrupted to the spawning grounds in order to insure future supplies of fish." In other words an adequate

breeding stock or spawning escapement each year must be permitted to pass the commercial fishery. What constitutes an adequate spawning escapement has not been clearly determined but it has generally been assumed that a return to the spawning grounds each year of as many spawners as were present in the preceding cycle year would tend to maintain the run at an even level of production.

Even the comparatively low percentage survivals noted above for the Cultus Lake and Karluk River areas would more than achieve this provision for on the basis of four thousand eggs per female sockeye a 0.1 or 0.2 per cent survival from the egg to adult stage would produce from two to four adults for each individual spawner. On this basis the commercial fishery could remove one or three adults, respectively, and yet leave the same spawning population as existed in the preceding cycle year.

There are only limited data available which suggest what relationships may now exist between the commercial fishing catches of sockeye and the spawning escapements. For Cultus Lake (Foerster, 1936, p. 35) in one year, 1932, marked fish recoveries indicated that those taken in the fishery approximately equalled those retaken at Cultus, or a 1:1 ratio, while in the second year, 1933, those taken in the catch constituted three times the number recovered at Cultus, or a 3:1 ratio. For the Columbia River, Rich (1942, pp. 131-137) has calculated a ratio of catch to escapement of 3.32:1 when based on numbers of fish or 4.11:1 when based on poundage.

Other investigators have endeavoured to ascertain the extent of the commercial fishing drain upon the total sockeye population of a river by means of tagging experiments. The results provide only a strictly minimum relationship and have shown for the Fraser River (MacKay, Howard and Killick, 1944) recoveries in fishing areas of 38 per cent in one series of taggings (Sooke) and 51 per cent in another (Johnstone Strait) and for the Skeena River (Pritchard, 1944) recoveries of 36.3 per cent of tags. These minimum estimates of the degree of exploitation, namely, ratios of catch to escapement of from 0.6:1 to 1:1 for the Fraser, 0.55:1 for the Skeena, might be contrasted with perhaps maximum estimates as calculated from the actual sockeye catches and the estimated spawning escapements as derived from spawning ground surveys. For the Fraser in 1943, the catch amounted to 51,090 cases (Alexander, 1944, p. J107) or 561,990 sockeye, at eleven fish per case, while the spawning ground surveys accounted for 118,691 individuals (Brennan, 1944, pp. 12-13) thus providing a ratio of catch to escapement of 4.8:1. For the Skeena, the 1944 catch amounted to approxi-

mately 800,000 sockeye while the spawning ground populations were estimated at around 250,000 fish, thus providing a ratio of catch to escapement of 3:1.

Between the commercial fishery and the spawning grounds of each river there exists a widely scattered but important Indian fishery which accounts for from fifty thousand to one hundred thousand sockeye each year and thus appreciably cuts into that portion of the total population which escapes the fishing grounds. It has not been considered in calculating the catch to escapement ratios.

If the Cultus Lake natural production record of 0.2 per cent survival of eggs to the adult stage is applicable to other sockeye areas it would appear that for each four returning sockeye the commercial fishery may remove three individuals and still leave the spawning escapement at the same level as before. Theoretically this ratio would provide optimum regulated production. If it were deemed that production of sockeye was lower than conditions could produce, a restricted fishery for a time which would provide a greater proportion of spawners would tend to increase production appreciably and effectively and place production and the commercial fishery on a higher level.

In Fig. 1 are plotted the numbers of returning adult sockeye produced per thousand eggs at varying rates of lake and ocean survival. If the average egg content per sockeye female is taken to be four thousand eggs or four thousand per pair of spawners (male and female), hence two thousand per individual fish, the returns in adult fish from each spawner would be twice the values shown in the figure. For example, if survival to migrant stage be four per cent and adult survival ten per cent, there should return eight sockeye adults for each fish spawning (two thousand eggs), therefore providing seven fish for the commercial fishery and one for the spawning escapement. If adult survival be twenty per cent, there would be a return of sixteen fish for each spawner, permitting the removal of fifteen fish by the fishery.

It is evident from the data of Fig. 1 that variation in per cent survival of migrants and of adults may very materially influence the quantity of fish produced by a spawning. The rates of increase are high and any reduction in the degree of exploitation by the commercial fishery should quickly, by increasing the spawning escapement, lead to higher production as a whole. There is obviously, however, a limit to the increase possible, due to the limited capacities of (1) the spawning grounds to hold the eggs, (2) the lakes to carry the young throughout their one or two year period of residence and (3) the ocean to maintain large populations of feeding fish. Therefore, while proper balancing of the catch

to escapement ratio may under certain conditions lead to greater production of sockeye, once the optimum degree of production per area is reached it can only be held at that approximate level by careful management. That is to say, that while for Cultus Lake with a presumed migrant survival of two per cent of eggs deposited and an adult survival of ten per cent of seaward migrants, there will be produced four sockeye for every one spawning, nevertheless if the fishery does not remove three out of each four but allows a greater spawning escapement, hence a greater potential production of migrants, still, this can only proceed to the level of the capacity of Cultus Lake to maintain growing young sockeye. Thereafter, production may be maintained at that level, all conditions continuing to be favourable.

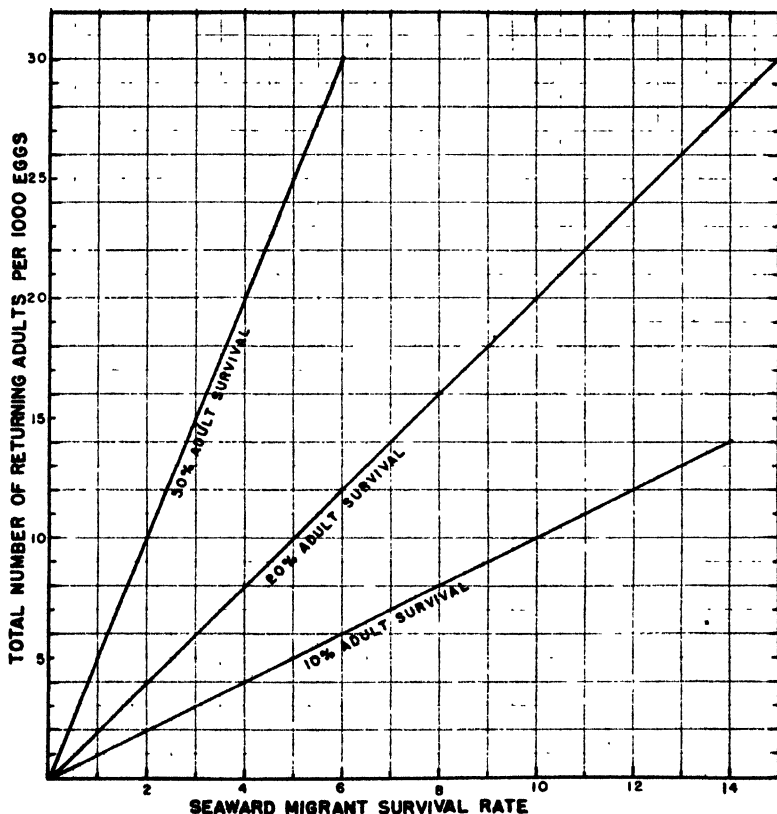


FIGURE 1.—Numbers of adult salmon produced per thousand eggs deposited as determined by survival rates (1) up to the seaward migrant stage, expressed as the percentage of eggs deposited, and (2) from seaward migrant to adult stage, expressed as the percentage of migrants proceeding to sea.

Barnaby (1944) has shown, however, that for Karluk River, where by legislative enactment the spawning escapement has been set at fifty per cent of the total return of salmon, i.e., a catch to escapement ratio of 1:1, the runs have shown no general increase. With calculated survival rates of one per cent to the seaward migrant stage and twenty-one per cent between migrant and adult stages four fish should return for each one spawning, allowing therefore a sufficient surplus over the prescribed 1:1 catch to escapement or 2:1 return to escapement ratios to provide an extensive and rapid increase in production. Yet no rise in the surplus occurred. No correlation was found between escapement and return and therefore it was concluded that mere increase in the numbers of fish allowed to proceed to the spawning grounds does not necessarily mean greater production in succeeding cycle years. From Barnaby's (1944) data it is found that in 1921, the escapement of 1,500,000 sockeye produced a return from the sea of 4,492,000, the escapement in 1922 of 400,000 spawners provided a return of 2,254,300 fish, whereas from 2,533,400 spawners in 1926 only 1,513,600 sockeye returned. These are probably extreme cases but they reveal, nevertheless, the great variation that may result in production from known escapements, the factors causing the variability apparently being associated with survival conditions on the spawning grounds or nursery lakes, hence influencing migrant survival.

The conclusion is reached, therefore, that while the size of the spawning escapement is important and, in order to maintain optimum production, there must be adequate seedings on all spawning beds, nevertheless the productive capacities of the producing areas are limited and regulation of the fishery to provide a highly favourable ratio of catch to escapement, e.g. 1:1 or 2:1 may only overtax the producing areas. The obvious objective in a progressive fisheries management scheme should be to keep the productive rate of the producing areas at a high level and make possible a high catch in relation to necessary escapement, e.g. 4:1, 6:1 or even 10:1.

It has already been remarked that whereas in the days before commercial fishing commenced the survival rate requirements to maintain the sockeye populations at optimum level did not require to be high—two eggs out of four thousand to reach the spawning grounds and reproduce, or 0.05 per cent—the removal of adult fish by the commercial fishery undoubtedly proved an appreciable drain and would require a higher survival rate to maintain the population. One would assume, however, that where many more eggs and fry are available than the spawning and nursery areas respectively would hold, a reduction in the

spawning population would produce less over-crowding and more favourable conditions for successful development of a greater percentage of the spawned product. In other words, if originally a million spawners produced a million returning spawners, with a mortality during the life cycle of 99.95 per cent, a half million spawners could readily produce a million returning fish by cutting mortality to 99.9 per cent through more favourable rearing conditions. If with a one half million spawning escapement, nursery areas were still severely taxed, resulting in heavy loss of young fish, reduction of the spawning escapement to one hundred thousand adults might still provide the one million returning fish by improving survival conditions and cutting mortality to 99.5 per cent of total eggs deposited.

The extent to which the survival rates will be influenced by conditions within the environment and vary inversely according to the relationship (*a*) of the total spawning effort (number of eggs deposited) to the capacity of the spawning beds or (*b*) of the total fry liberation to the capacities of the nursery lakes, is not known. If production of young fish be limited and controlled by the capacity of the nursery area, it would be assumed that the survival rates during development of the young fish would so vary that there would be more or less a constant yield from the nursery waters. If the fry liberation were very heavy, survival would be low; if the fry in-go were light, survival would be high.

The available evidence fails to support such an hypothesis for at Cultus Lake (Foerster, 1938a) when the seeding (number of eggs available for deposition) varied from 17,500,000 to 250,000,000 eggs in different years, the percentage survival to smolt stage varied only from 1.13 to 1.05 to 3.23 per cent. Even when the efficiency of propagation to the smolt stage was calculated on the basis of the number of males present (to correct for unequal sex ratios since females predominated) the percentage survival data were 2.86, 2.25 and 3.47 per cent respectively. Moreover when fry liberations were conducted (1926 - 5,900,000; 1929 - 9,100,000; 1932 - 4,800,000) the percentage survivals were 5.83, 3.85 and 2.81 per cent, respectively. In other words no inverse correlation was found which would tend to indicate a variation around a mean capacity level for the lake, influenced primarily by supplies of eggs or young fish present.

Nevertheless, while the variable nature of the lake's productive capacity in relation to populations of fish being nourished does not appear to apply to percentage survival rates, contrary to what one might expect,

it does become revealed in the weight of fish produced, i.e., size of migrants. For the years 1927 to 1935 a coefficient of correlation (weight of fish to population) of -0.815 was obtained (Foerster, 1944, p. 276) and the relationship shown of each year's lake production, as indicated by the mean weight of migrants, to a production band of six to eight thousand kg. On the basis of the relationship trends obtained, it was suggested that Cultus Lake could probably not support a lake population of sockeye larger than that required to produce a seaward migration of three or at most four million yearling migrants, unless radical changes were made in the lake's economy, e.g. reduction in numbers of predator and competitor fishes.

It is apparent that significant increases in survival rate of young sockeye occur only when the upper limits of the productive capacities of the spawning or nursery areas are approached. When the populations are at a relatively low level other factors intervene and complicate the situation. The general relationship may perhaps be more clearly shown for Karluk River (Barnaby, 1944, p. 259, Fig. 3), where there are indications that relatively small escapements produce greater returns, i.e., permitted a higher rate of production, than large spawning runs.

Under such circumstances, consequently, the objective should be to so regulate the fishery that there be permitted to reach the spawning areas sufficient spawners to seed the spawning beds fully and populate the nursery lakes. Thereupon specific lake management projects may be undertaken to increase spawning reaches and ensure more favourable environmental factors for successful development.

For other species of Pacific salmon the data are less complete. For *O. tshawytscha*, chinook or spring salmon, Rich (1942) reports for the Columbia River in 1938 that over eighty per cent of the spring run and between sixty and seventy per cent of the main fall run were taken in the commercial fishery. He states that "It seems reasonably certain that, at least for the spring run of chinooks on the Columbia, the escapement is well below the level that would provide the maximum sustained yield." For the coho, *O. kisutch*, the chum, *O. keta*, and the pink salmon, *O. gorbuscha*, estimates of the relationship of catch to escapement are not available but Pritchard (1939) has reported that "of the eggs carried by a spawning run of pink salmon, 6.9 to 24.0 per cent may develop to migrate seaward as fry" and tests of coho production in two streams tributary to the Cowichan River (Foerster, 1945, p. 44) showed variations of from 11.8 per cent to 40.0 per cent in production of seaward migrating fry from known egg depositions.

Increasing the productive capacities of spawning and nursery grounds

The extent to which natural production of the various species of Pacific salmon can be appreciably stepped up depends somewhat upon the spawning habits of the species and the length of time spent in fresh water. Its importance lies not only in restoring runs to their former abundance but in increasing the productivity of the areas to higher levels.

For sockeye salmon, which spend at least one year in fresh-water lakes, it seems likely that spawning ground improvement is perhaps of less significance than the control of predators in the lake. Experiments designed to reveal the effect of removal of predators and appreciable reduction in resident populations thereof have been conducted at Cultus Lake (Foerster and Ricker, 1941) and it was indicated that survival rates of young sockeye in Cultus Lake were increased from 250 to 400 per cent of their previous value. It is apparent that reduction and control of predator fish populations may increase directly and most appreciably sockeye salmon production.

Improvement occurs not only in rate of survival or rate of production but also in relative size of the fish. Comparisons made of the sizes of seaward migrants resulting from large populations in Cultus Lake before and after predator control was initiated (Foerster, 1944) show that the inverse correlation of size of fish to population density is very appreciably changed when predators are removed and much larger migrants result. If, as has been intimated above, there is a relationship between productive capacity of an area and the weight of fish produced, the larger numbers of migrant sockeye of larger size obtained under conditions of predator control would suggest an appreciably increased productive capacity for the area, hence considerably more sockeye of small size than the area previously produced.

Another important feature limiting natural production of salmon is the presence in the rivers of obstructions to the ready ascent of the fish. While they may not present a complete blockade, nevertheless their delaying action may have deleterious effects and their discovery and improvement give assurance of unimpeded arrival of the fish on the spawning grounds.

For the pink and chum salmon, those species that frequent chiefly coastal streams and that spawn in the lower reaches of the rivers, stream control and maintenance of suitable spawning grounds are important features. In the spawning areas freshets in the fall are frequent, particularly, it would appear, in burned-over deforested areas, and tend to scour out the eggs and alevins. Low water conditions occur in the spring and in many areas the redds are left high and dry or the newly-

hatched fry isolated in shallow pools subject to easy destruction by birds, etc. River channels may change and leave whole spawning areas a total loss. In such areas suitable stream control measures can be particularly effective and at the present time a study of feasible methods of improvement is being made on the east coast of Vancouver Island by the Fisheries Research Board.

From studies of salmon spawning in the Amour and Kamchatka Rivers, Kusnetzow (1928) computed the losses of eggs and alevins in normal chum salmon spawning as approximately thirty per cent. To this is added a further twenty per cent for freezing and drying. Artificial propagation had been considered as a means of avoiding many of these losses but the conclusion reached was that "it would be more rational for the restoration and the maintenance of the stock of salmon to take as a base the principle of protecting natural propagation under natural conditions and consider artificial propagation only as a necessary addition to the former."

Where, as may be found in many streams of British Columbia, the pink and chum salmon runs are extremely variable, and, in many cases, a fraction only of their former size, careful attention to spawning ground conditions and improvement of these together with adequate control of the stream flow may very appreciably increase production of young salmon and reinstate and stabilize the industry on a much sounder basis.

SUMMARY

In undertaking management programmes for optimum production of Pacific salmon to allow maximum commercial exploitation consistent with adequate perpetuation of the species the existing state of the fishery must be analyzed and the limiting factors properly evaluated.

Declines in the salmon fisheries are usually attributed to either over-fishing or decreased production of young fish on the spawning and nursery grounds.

For sockeye salmon data available on efficiency of reproduction indicate that on the basis of 0.2 per cent survival from egg to adult stages four adults return for each previous spawner, thus permitting the removal of three individuals by the commercial fishery and leaving one to maintain the run. At these rates limitation of the fishery should soon increase the stocks of salmon to the capacity of the spawning and nursery grounds and retain it at that level, but data from Karluk River, Alaska, experiments reveal that for optimum production the spawning

escapements should be regulated according to the capacities of the producing areas.

For sockeye salmon, from forty to seventy-five or eighty per cent of the runs are taken by the commercial fishery; for spring or chinook salmon on the Columbia River, from sixty to eighty per cent of the runs are removed; but for the other three species of Pacific salmon catch to escapement records are not available. Figures regarding production of fry have been obtained in certain cases and show considerable variability from year to year.

With reference to increasing the productive capacities of salmon-producing areas experiments have shown that control of predators is an important problem for sockeye, probably also for the other species, and that for pink and chum salmon stream control and adequate provision of spawning grounds with controlled water flows may materially increase production of young.

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ON THE HYDROID *DAHLGRENELLA FARCTA* MILES

By C. McLEAN FRASER, F.R.S.C.

IN the summer of 1936, at the Mount Desert Island Biological Laboratory, working with Dr. Ulric Dahlgren of Princeton University, Samuel Stockton Miles (1937) collected several specimens of a species of hydroid of more than usual interest, which he named *Dahlgrenella farcta* (n.g., n.s.). These specimens were obtained in muddy bottom at a depth of 40-60 feet, in Frenchmans Bay, off Salsbury Cove, in association with *Acaulis primarius* Stimpson and *Corymorpha pendula* Agassiz. The species was reported with little detail (1937) in the Bulletin of the Mount Desert Island Biological Laboratory; a more detailed description was given in the Biological Bulletin (1937).

Instead of carrying through his observations and making further use of the material he had prepared, Miles entered Johns Hopkins University Medical School. He graduated in Medicine in time to offer his services when war was declared in 1941, and he was sent to the Solomons, where he met his death on Guadalcanal at the hand of a wounded captive, to whom he was giving remedial treatment.

On the suggestion of Dr. Dahlgren, Mrs. Miles offered to look up any material and notes bearing on *Dahlgrenella* that her husband had left behind, to send to me if I wished to make any use of them. The offer was gladly accepted and in due time the notes and the material arrived. An examination of the whole mounts and the series of sagittal sections brought to light some features that may have been observed but had not been recorded in the published papers. Possibly the best way to indicate the value of Miles's work and to show appreciation of my obligation to Mrs. Miles and Dr. Dahlgren, is to give an account of these special features, while giving full credit to Dr. Miles for making the examination possible.

Dahlgrenella farcta is a very interesting species, in some respects, unique, but it does not stand so much alone as Miles surmised, for several of its unique characters are shared with its nearest relative so far described, *Hypolytus peregrinus* Murbach, of which evidently he had no knowledge. Although the two species have enough differences to be placed in different genera, more of the unique features are common to both. They are the sole representatives at present of the family *Hypolytidae*. If Miles had been familiar with Murbach's excellent description of *H. peregrinus* (Murbach, 1899), it would have

eased his investigation very materially, and might have suggested certain desirable observations that could have been made satisfactorily only on living specimens.

Since only one species in each of the genera, *Hypolytus* and *Dahlgrenella*, has been described, it is difficult to decide which of certain characters are generic and which specific. A detailed comparison of the two species will indicate the resemblances and the differences sufficiently to distinguish most of these characters, as well as those which serve to isolate the family *Hypolytidae* from other families, these being the characters that are more unique.

The two species do not agree in the nature of their habitat; *D. farcta* lives in muddy bottom in 40-60 feet of water, while *H. peregrinus* may be living freely in the water near the surface or temporarily attached to eelgrass or other solid objects at or near the surface. They agree in that neither of them is permanently attached to the substrate; each may move about to some extent, but for most of the time each remains temporarily attached by means of a hyaline perisarc, of somewhat hardened mucus, any part of which may be used for the purpose. The thin, perisarcial sheath may extend beyond the coenosarc. One might suppose that this is a family character, and it may be so, but it is not restricted to the *Hypolytidae*, because something similar is present in *Acaulis primarius*, and the *Acaulidae* is not very closely related to the *Hypolytidae*. According to Miles, the frustules scattered over the surface of the hydrocaulus in *D. farcta* may be used for temporary attachment as well. Evidently no frustules were observed by Murbach in *H. peregrinus*.

D. farcta, 5 mm. to the end of the coenosarc, is not so large as *H. peregrinus*, 10-15 mm., but the hydrocaulus of each is flexible; the free proximal end is usually curved. The thin, perisarcial sheath that covers the hydrocaulus is torn off or torn apart readily, but it can be quickly and readily renewed. Apparently this destruction and rehabilitation is more or less continuous when the zooid is in ambulatory motion. Swimming or floating freely in the water may serve for variety.

In both, the body of the hydranth is nearly tubular, but in *D. farcta* it is of greater diameter in the proximal portion; in both there is some tapering from the distal end of the hydrocaulus to the proximal, free end. The body of the hydranth, or what appears externally to be this, is clearly marked off from the distal end of the hydrocaulus by a sudden diminution in the thickness of the ectoderm; this is so evident that the proximal portion of the wall of the hydranth appears as a definite collar.

The filiform tentacles appear in two whorls in each, and there are no capitate tentacles. There is a maximum of sixteen basal and eight oral tentacles in *D. farcta*; fourteen proximal and ten distal in *H. peregrinus*. The proximal tentacles are long and the distal short, shorter in *D. farcta* than in *H. peregrinus*. They all appear stout because of the rings or spirals of nematocysts which are quite close to one another, especially when the tentacles are contracted. The basal whorl of tentacles in both species is some considerable distance away from the base of the hydranth body, a distance equal to about one-third of the length of the hydranth; the distal whorl surrounds the mouth quite closely.

In *D. farcta*, frustules are scattered over the surface of the hydrocaulus; these are like miniature tentacles, with a single layer of ectoderm cells (there are no nematocysts present), and a core of large endoderm cells. As the frustule grows out, it pushes the perisarc with it, or ahead of it, so that it is always surrounded with this perisarc. According to Miles, these frustules may be used for attachment in the same way as the perisarc of the entire hydrocaulus. If the time of attachment is prolonged, the ectoderm and the endoderm of the frustule may disintegrate leaving the frustule sheath to retain the attachment. Murbach mentions no such structures in *H. peregrinus*.

In both, the terminal mouth opening is very small. In *D. farcta*, the gastric cavity is elliptical, broadly elliptical in preserved specimens. It extends proximally for some distance into the part that, judging from the outside, would be considered to be the hydrocaulus, before it narrows to form the regular, tubular cavity of the more slender part of the hydrocaulus. The endoderm in the wall of this cavity is highly specialized for that of a coelenterate; the surface is as much convoluted as the mucosa of the small intestine of a mammal, and bears much resemblance to it. Proximal to the enlarged part of the cavity, in the tubular portion, the endoderm is not so specialized. This must be quite different from that of *H. peregrinus*. Murbach has not described the latter in detail but he says: "Just below the point where the aboral tentacles are attached there is an enlargement of the digestive cavity, looking like a deeply pigmented band running across the coelenteron." There is no sign of such enlargement in *D. farcta*.

A difference of definite, generic value appears in the gonosome. In *D. farcta*, the gonophores produce free medusae; in *H. peregrinus*, they produce fixed sporosacs. In *D. farcta*, a portion of the wall of the gastric cavity is pushed outward to form a somewhat elongated,

hollow axis, at the terminus of which the first medusa bud appears; lateral outgrowths of the axis give rise to five or six other medusa buds later, all on short pedicels, the latest buds nearest to the base of the axis. The oldest bud observed by Miles had four well-developed tentacle bulbs, but there was a tentacle on only one of these. At present, there is no means of knowing if other tentacles develop later. Four clusters of these medusa buds are developed from the hydranth body wall just distal to the aboral tentacles.

In *H. peregrinus*, the gonophores appear in the same position relative to the aboral tentacles. Each gonophore gives rise to a single, large, elongated oval sporosac supported on a short pedicel. When fully developed it may be 2 mm. or more in length. From the pedicel there is a short, slender diverticulum that appears to be homologous with one of the lateral pedicels in *D. farcta*. When the terminal sporosac developed to such a size, apparently the lateral sporosacs disappeared, the last one to do so leaving the pedicel as a vestige. The number of peduncles is reduced from four, as in *D. farcta*, to three, two, or one in *H. peregrinus*.

A type of asexual reproduction appears in both species which is quite unlike that anywhere else among the hydroids; it is the unique, family character. A constriction appears in the hydrocaulus near the free end; this increases until an ovoid body is separated off from the main part of the hydrocaulus, with the length two or more times the breadth. Murbach called such a body a "blastolyte," and Miles, an "ovoid body"; it remains within the sheath for some time. This procedure may be repeated. Murbach observed several such bodies formed in *H. peregrinus*, but never more than two of them remained in the sheath at one time. Miles, on the other hand, observed as many as twenty bodies in the one sheath in *D. farcta*. According to Miles, fission may take place occasionally in one of these ovoid bodies to form two such bodies. Each body develops into a normal zooid. Early in the development it breaks through the sheath to become free. Both observers made an attempt to follow the development through, but in neither case is the picture very complete. An examination of the preserved specimens of *D. farcta* did not help matters. Further observations on the living specimens are needed.

In all of the bodies observed by Murbach, the polarity was the same as in the parent zooid. In what appears to be the normal development, the body soon becomes ovate, the larger end being that towards the main hydrocaulus; soon that end becomes the hydranth body; some of the tentacles appear as knobs before the mouth breaks through. The narrow end becomes the hydrocaulus and the perisarc

is soon secreted. In some instances, however, the procedure was different. The ovoid body did not become ovate; instead, a decided, lateral thickening developed, which extended to become the hydranth end of the young zooid; the two ends gradually came together and fused to form the hydrocaulus, and then the development proceeded as in the normal hydroids. Early in the development, after the zooid begins to take form, it breaks through the perisarcial sheath to become free in the water.

In *D. farcta*, Miles found it "extremely difficult to determine the orientation of the bodies." His conclusions may be given in his own words:

It may be of some significance in the interpretation of this mode of reproduction that the young individuals appear to develop oriented oppositely from the parent. The hypostomata of the young in most cases, point posteriorly in the hydrorhiza. Since two individuals pointed in the same direction may develop from a single body constricting equatorially, it is evident that the orientation is not dependent upon the location of the constriction. The possibility of the presence of an axial gradient should be investigated. However, the bodies become motile early and possibly reverse their positions by their movements. This possibility seems remote since one can observe in the position and structure of the sheath no conditions which might cause the bodies to point uniformly in a direction in which they had not developed.

However, in a series of 17 specimens kept on a glass slide over a period of 4 days, 25 bodies were observed which apparently pointed in the opposite direction from the parent, while 2 seemed to point in the same direction. Of 7 other bodies whose orientation was more questionable, 6 seemed opposite from the parent and 1, the same. In only 1 case was there evidence that the body had reversed its position.

Evidently Miles observed no instance in *D. farcta* where the hydranth grew out of the ovoid body from a lateral thickening of the body as in the exceptional cases observed by Murbach in *H. peregrinus*.

In the material forwarded by Mrs. Miles, there is a fine series of sagittal sections of a *Dahlgrenella* zooid. These sections show several rather special histological features which should be described even although there is no detailed, histological description of *H. peregrinus* with which to compare them.

As the perisarc is non-cellular, it shows no distinctive, histological features. The ectoderm is separated from the endoderm throughout by a well-defined, but thin layer of mesoglea, that shows in section everywhere as a distinct line. The ectoderm varies materially in different regions. Between the mouth opening and the base of the oral tentacles, it is mainly one cell thick, with the cells little specialized, although in places there are nematocysts present. Between the oral and the aboral tentacles, and extending further to the margin of the

collar, the cellular structure is much similar, but the cells appear in several ill-defined layers, scarcely regular enough to be described as stratified epithelium. Nematocysts are quite numerous. Immediately below the margin of the collar there is an abrupt decrease in the thickness of the ectoderm to a single layer of columnar cells. This modified ectoderm extends for a distance nearly equal to the distance between the base of the aboral tentacles and the margin of the collar, after which in the remainder of the wall of the hydrocaulus there is but a single layer of simple, unspecialized cells.

In about three-fourths of the wall of the main gastric cavity the endoderm is of considerable thickness and the surface is strongly convoluted, so that it resembles, in general appearance, the mucosa in some parts of the small intestine in the mammals. To make the likeness more noticeable, in some of the convolutions there are cells that look much like mammalian, serous cells, and in others, cells that look like mucous cells. These latter may in some cases even resemble the more highly specialized goblet cells. In the remainder of the cavity the endoderm is little differentiated, and laterally forms a thin layer. Toward the base and extending along the central cavity of the more slender part of the hydrocaulus, it may be in several indistinct layers entirely unspecialized. There is no demarcation where the endoderm narrows to pass into the tubular portion of the hydrocaulus.

The gastric cavity is projected into the peduncle of the gonophore, but the layer of endoderm here becomes thin. The cavity does not extend into the tentacles, where the endoderm forms a vacuolated core, and the ectoderm is thin but is well supplied with nematocysts.

It is of some interest to compare the more minute structure of *D. farcta* with that of *Acaulis primarius* Stimpson (Fraser, 1924), since this species is associated with *D. farcta* in muddy bottom in Frenchmans Bay, and, like it, has no permanent attachment to any substrate. Not only is the hydrocaulus unattached but it is so much reduced as to be almost or entirely vestigial. The hyaline sheath that surrounds the hydrocaulus is comparable to that of *D. farcta*. The ectodermal layer is of much the same thickness throughout, corresponding to the thin regions of *D. farcta*. The gastric cavity is tubular with no part of it much enlarged; the cavity does not extend into the hydrocaulus. In the whole wall of the cavity the endoderm is thickened; the surface is irregular but can scarcely be said to be convoluted; there is little sign of the high degree of specialization. The whole core of the hydrocaulus is made of large-celled endoderm, so that it may be described as vacuolated. The vacuolated core is

separated from the base of the gastric cavity by a layer of columnar endoderm.

Although in the *Hypolytidae* there are at present but the two monotypic genera, *Hypolytus* and *Dahlgrenella*, making it difficult in some cases to decide whether a character is generic or specific, there are enough clear-cut characters to leave no doubt that the family *Hypolytidae* is distinct from other gymnoblastic families, and that *Dahlgrenella* is a genus distinct from *Hypolytus*.

The characters that separate the family *Hypolytidae* from other families in the same group of gymnoblasts with an oral and an aboral whorl of filiform tentacles are: (a) The proximal end of the hydrocaulus has no permanent attachment to any substrate. Temporary attachment may be provided by making use of the hyaline perisarcal sheath which itself may be but temporary, since it may be readily torn or removed but is soon replaced by further secretion by the ectoderm of mucus or mucus-like material. (b) Besides the regular sexual reproduction, there is a distinctive type of asexual reproduction. Transverse fission of the hydrocaulus takes place not far from the free end to separate off an ovoid body that develops into a normal zooid. In the early development it remains within the sheath, but soon it breaks through to become free in the water, where the later development takes place until the perisarcal sheath is formed and temporary attachment may take place.

The most distinctive, generic difference between the two genera appears in the method of sexual reproduction. In *Hypolytus*, the gonophores produce fixed sporosacs, and in *Dahlgrenella*, free medusae. It is quite possible that other differences between *H. peregrinus* and *D. farcta* are generic as well.

SUMMARY

Dahlgrenella farcta Miles bears much resemblance to its nearest relative, *Hypolytus peregrinus* Murbach. The most evident generic difference appears in the sexual reproduction: in *Hypolytus*, the gonophores produce fixed sporosacs, and in *Dahlgrenella*, they produce free medusae. There are many other differences, but as each genus at present is monotypic, it is not easy to decide whether the differences between the two species are generic or specific.

The two genera are so similar that they must be placed in the one family *Hypolytidae*, the most distinctive characters of which are the free proximal end of the hydrocaulus, provided for much of the time with a readily removable and renewable perisarcal sheath, by the

use of which temporary attachment may be made, and the special type of asexual reproduction by transverse fission of the hydrocaulus near its free end, to form an ovoid body which develops into a normal zooid.

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EXPLANATION OF PLATE I

Dahlgrenella farcta

FIGURE 1.—Sagittal section through zooid showing position of oral and aboral tentacles, and the varying thicknesses of the endoderm and the ectoderm (x45).

FIGURE 2.—Distal portion of sagittal section through zooid, and through the gonophore peduncle (x45).

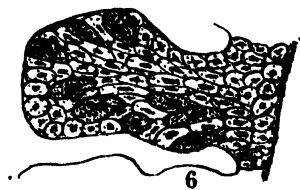
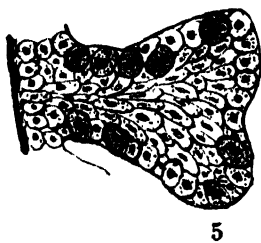
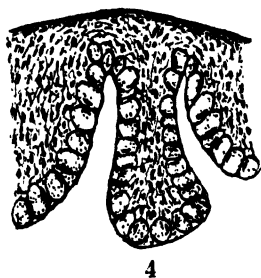
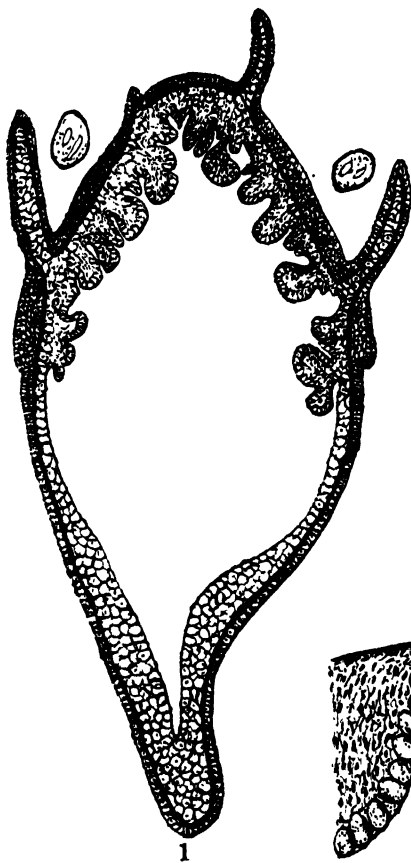
FIGURE 3.—Sagittal section through a portion of the ectoderm to show the stratification in the collar and the simple, columnar ectoderm adjacent to it (x200).

FIGURE 4.—Section through a convolution of the endoderm of the gastric cavity near the mouth opening showing cells resembling mammalian, serous cells (x200).

FIGURE 5.—Section through an endoderm convolution showing cells resembling mammalian, mucous cells (x200).

FIGURE 6.—Section through an endoderm convolution showing cells resembling mammalian, goblet cells (x200).

PLATE I



COMPARATIVE CHEMISTRY AS AN AID TO THE SOLUTION
OF PROBLEMS IN SYSTEMATIC BOTANY

By R. DARNLEY GIBBS, F.R.S.C.

INTRODUCTION

MOST taxonomists would agree with Gilmour's (1937) statements that: "... a natural classification is one founded on attributes which have a number of other attributes correlated with them, while in an artificial classification [as in a "Key"] such correlation is reduced to a minimum. . . "; and that: "The categories and nomenclature of traditional taxonomy should be confined to the most natural classification possible in the existing state of knowledge of any particular group, on whatsoever attributes it may be based, and such a classification would be the most generally useful for a great variety of purposes, both scientific and non-scientific."

The attributes upon which plant taxonomy is chiefly based are those of morphology—visible features which may be studied with some facility—and no one would wish it otherwise, but when even a casual perusal of recent "systems" of taxonomy reveals fundamental disagreements among the most skilled specialists, it becomes obvious that further attributes must be considered in order to resolve these difficulties.

In some cases a study of anatomy and cytology has been of assistance, but anatomy and cytology are in a sense more detailed morphology and all three are the outward or at least the visible signs of inward chemical and physical differences. It would seem, then, that comparative chemistry should be of the greatest help and that the really valuable advances in taxonomy may well be made by men trained in two major fields—in taxonomy and in plant biochemistry. This is not a new idea and there are many parallels in the other sciences. Men with honours degrees in physics sometimes turn to medicine, become fully qualified doctors, and apply their training in physics to the problems of neurology. This is often much better than the co-operation of two specialists and almost infinitely better than the sometimes pitiful efforts of the expert in one field, who trespasses into another without really understanding it.

Until now the major workers on plant systematics have been disappointingly aloof and one looks in vain in their books for any reference to the not inconsiderable work in comparative chemistry that is already on record in the literature. The present paper attempts to summarize

a little of this work and to apply it to taxonomy. It is designed to be the first of a series on this same topic.

In this communication we shall ignore altogether the much disputed serological methods which are based upon protein differences in related plants. The reader is referred to the papers by Chester (1937) on this topic. We shall ignore, too, the very extensive works by Reichert (1913, 1919) on starches and by Carles (1935) on the carbohydrates of the Iridaceae; works which have received scant notice from the taxonomists. Further examples and the early history of the subject may be found in a paper by Jaretsky (1928). We shall confine our discussion here to the distribution of nitrogenous anthocyanins, the anthocyanins of *Tulipa*, the fats of the Flacourtiaceae (or of two tribes thereof), and the comparative chemistry of the Cupressaceae, topics which illustrate the use of chemistry as a weapon in taxonomy.

NITROGENOUS ANTHOCYANINS

Of anthocyanins generally we shall say little, though a detailed consideration of their distribution (which in spite of the extensive work of Robinson and his co-workers is still very incompletely known) might well yield results of considerable significance. The nitrogen-containing anthocyanins, however, may receive some attention. Sir Robert Robinson, in a recent paper (1942), says: "Nitrogenous anthocyanins have been found in five orders only,¹ namely Caryophyllales, Chenopodiales, Lythrales, Thymelaeales, and Cactales. It is probable, therefore, that the Cactales are not so remote from the four orders first named as is tentatively suggested by Hutchinson in his Families of Flowering Plants (1926)." Anyone reading only this paper would assume, as did the present writer, that Sir Robert had missed Hutchinson's statement on p. 17, where he says of the Cactales that they might alternatively be placed next to the Ficoidaceae (that is, in or next to the Caryophyllales). In an earlier paper, however, Lawrence, Price, Robinson and Robinson (1939) take full notice of Hutchinson's uncertainty and yet sum up the situation thus: "The fact that the nitrogenous anthocyanins are found only in these five orders would in itself have little phylogenetic significance. However, many systematists differ from Hutchinson on morphological grounds in his placing of the Cactales, and taken in conjunction with this the distribution of

¹Robinson, apparently, does not consider the pigment nudicaulin of *Papaver nudicaule* (and perhaps of *P. alpinum* and *Meconopsis cambrica*) as a nitrogenous anthocyanin, though it does contain nitrogen, and was thought to be a flavylum compound (Price, Robinson and Scott-Moncrieff, 1939).

the nitrogenous anthocyanins indicates that the Cactales are closely related to the Caryophyllales, Chenopodiales, Lythrales and Thymeleales." This would give us the scheme shown in Fig. 1.

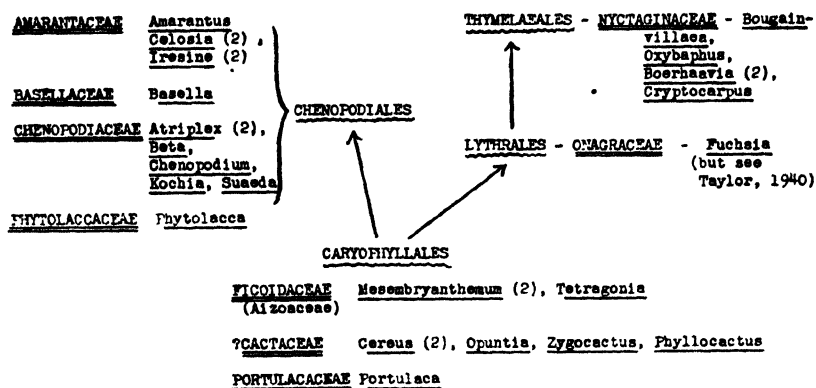


FIGURE 1.—Plants known or previously reported to contain nitrogenous anthocyanins arranged according to Hutchinson (numbers in brackets indicate the number of species investigated).

Some doubt, however, has been thrown upon the occurrence of these compounds in the Lythrales. Kryz (1920) reported that the berries of *Fuchsia discolor* "und Verwandte" contain anthocyanins having the same reactions as those of the red beet-root, but Taylor (1940) quotes Price, without reference, as failing to find nitrogenous anthocyanins in *Fuchsia* and says: "no other member of the order to which *Fuchsia* belongs (Lythrales) has been found to contain a nitrogenous anthocyanin. . . . Hence the present position is that the order Lythrales, placed by Hutchinson as leading from an order in which nitrogenous anthocyanins occur to another in which, as far as is known, they are invariable, itself contains no such pigment. . . . from a chemical point of view Hutchinson's sequence is unsatisfactory."

A consideration of the position of the Nyctaginaceae is highly pertinent in this connection, and the occurrence of nitrogenous anthocyanins in the family is of great interest. Hutchinson places it in his order Thymelaeales which he regards as derived from the Caryophyllales through the Lythrales (Fig. 1). Its position is by no means clear, however, and Maheshwari (1930) says, after a study of *Boerhaavia diffusa*: "Hutchinson has separated the family Nyctaginaceae from the closely allied families Amaranthaceae, Phytolaccaceae, Basellaceae, Chenopodiaceae, etc., and has put it and three other families, the

Geissolomataceae, Thymelaeaceae, and Penaeaceae in a separate order, the Thymelaeales, which is supposed to be derived from the Lythrales. The close anatomical similarities between the families Nyctaginaceae, Amaranthaceae, Chenopodiaceae, and Phytolaccaceae, seem to constitute a strong argument against this view." Joshi and Rao (1934) also report evidence for the close relationship of Nyctaginaceae with Phytolaccaceae, maintaining that the gynoeceum of the former is exactly similar to that of some members of the Phytolaccaceae like

Rivina.

In the Engler and Prantl system the family Nyctaginaceae is placed in the order Centrospermae—essentially Hutchinson's Caryophyllales. The families Geissolomataceae, Thymelaeaceae and Penaeaceae, on the other hand, are classed with the Myrtiflorae. It is obviously highly desirable to secure further facts on the chemistry of these groups, and the present writer is searching the literature for such information as is available. For the present it would seem that the existence of nitrogenous anthocyanins in the Nyctaginaceae would support the inclusion of the family in the Centrospermae (Caryophyllales) rather than in the Thymelaeales, especially as such a position would remove the Thymelaeales from our list and would leave only the order Centrospermae (of Engler and Prantl) with seven of its twelve families known to contain nitrogenous anthocyanins, and the order Opuntiales (Cactales) of uncertain position.

It is interesting to note that Engler (1925), in an article on this last order in the second edition of *Die Natürlichen Pflanzenfamilien*, saw a relationship between it and the Centrospermae and considered that the two orders might have been derived from related polyandric ancestors:

Eine direkte Abstammung der Cactaceen von der Aizoaceen oder Portulacaceen scheint mir daher noch nicht ganz sicher, wohl aber möchte ich wegen der in diesen Familien sich findenden Anklänge annehmen, dass sie von einander nahestehenden polyandrischen Vorfahren herzuleiten sind, welche zur Zeit irgendwelcher Überbrückung Südafrikas mit Südamerika in diesen beiden Kontinenten und den Zwischenländern existierten, von denen aber der Ast der Cactaceen in Amerika mit grosser Zähigkeit die azyklische Anordnung der Blütenhüllblätter und der Stam., sowie eine grössere Zahl von tief in der Blütenachse Karpellen bewahrend eine ausserordentlich reiche Entwicklung erreichte, während Aizoaceen, Phytolaccaceen und Portulacaceen, die ersteren namentlich in der Alten Welt, in ihren Blütenverhältnissen eine grosse Wandelbarkeit durch Reduktion und zyklische Anordnung erreichten.

He recognized as an alternative, that the Cactaceae might even be placed *in* the order Centrospermae as its first family. Engler is by no means alone in his belief in a relationship between the Cactaceae and the Aizoaceae (Ficoidaceae) for Bentham and Hooker (1862-7) placed

the Cactaceae and the Ficoidaceae together in their cohort Ficoidales. It should be remarked, however, that Bentham and Hooker separated the Ficoidales widely from the Caryophyllineae and Curvembryeae which contain the other families which have nitrogenous anthocyanins. Croizat (1944-5) is most emphatic in stating that the Cactaceae and Aizoaceae are *not* closely related. He believes that the Cacti are nearer *Punica* (in the Myrtiflorae of Engler and Prantl).

If, for the moment, we accept Engler's guess as to the derivation of the Centrospermae and the Opuntiales from common ancestors, we have the arrangement shown in Fig. 2 which seems much better than that of Fig. 1. We might guess, in turn, that these ancestors also had nitrogenous anthocyanins! It would be interesting, in this connection, to study the, supposedly, most primitive plants of both orders and to contrast them with the most advanced. Croizat's views, too, might be checked by a knowledge of the comparative chemistry of *Punica* and the Cactaceae.

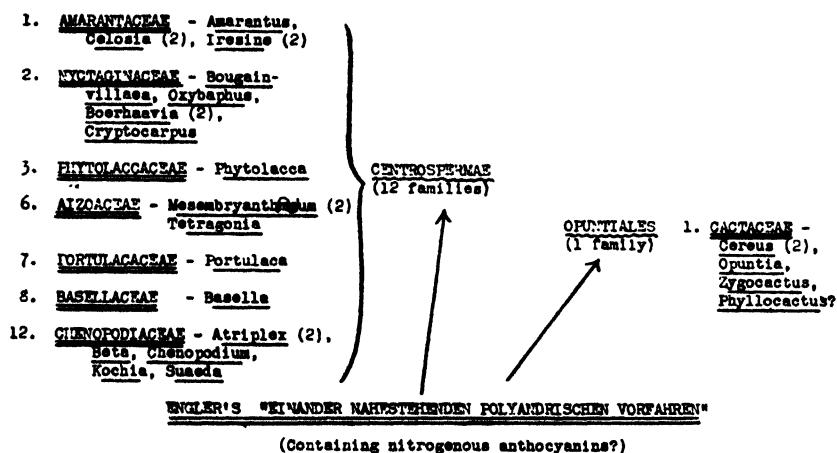


FIGURE 2.—Plants known to contain nitrogenous anthocyanins, arranged according to Engler (1925). Data chiefly from Lawrence, Price, Robinson and Robinson (1939) and Taylor (1940).

ANTHOCYANINS OF *Tulipa*

In the discussion above we have been considering families and orders and their relationships and placing. As a contrast the distribution of anthocyanins within a single genus—*Tulipa*—may be considered. Sir A. Daniel Hall (1940) has monographed this genus and has included data on the anthocyanins contained in the beautiful flowers of these popular plants. Further analyses are reported by Beale, Price and

Sturges (1941). Hall divides the genus into two sections, Eriostemones and Leiostemones, and these in turn into sub-sections as follows:

- A. ERIOSTEMONES—with hairs on bases of filaments.
 - I. Australes—about 10 species.
 - II. Saxatiles—about 8 species.
 - III. Biflores—about 4 species.
- B. LEIOSTEMONES—no hairs on filaments—not a natural sub-genus?
 - I. Clusianae—about 11 species.
 - II. Gesnerianae—about 33 species.
 - III. Oculus-solis group—about 15 species.
 - IV. Eichleres—about 8 species.
 - V. Kolpakowskianae—about 8 species.
- C. SPECIES STANDING ALONE—about 2 species.

When the classification given above is checked by anthocyanin distribution (excluding the basal blotch on the perianth segments) it is found that at least the division into the groups Eriostemones and Leiostemones is confirmed. Hall's statement that the members of the Leiostemones "do not constitute a natural sub-genus" is also supported, the Eichleres differing from the others in having no cyanidin pigments. These facts are shown in summary form in Table I.

TABLE I

Groups of Species	No. of spp. examined	Nos. of species containing the anthocyanins indicated					
		**D.3-PG	D.di	Cy.3-PG	Cy.di	P.3-PG	P.di
A. ERIOSTEMONES							
I. AUSTRALES	*3/10	2	2	1	0	0	0
II. SAXATILES	6/8	2	4	2	0	0	0
III. BIFLORES	0/4	—	—	—	—	—	—
B. LEIOSTEMONES							
I. CLUSIANAE	6/11	0	0	3	0	6	0
II. GESNERIANAE	1/33	0	0	1	0	1	0
III. OCULUS-SOLIS	11/15	0	0	7	3	11	0
IV. EICHLERES	4/8	0	0	0	0	4	1
V. KOLPAKOWSKIANAE	1/8	0	0	1	0	1	0
C. OTHER SPECIES	2/2	0	0	2	1	1	0

TABLE I.—Distribution of anthocyanins within the genus *Tulipa*. (*3/10 means 3 spp. examined out of a total of 10 in the group. **D.3-PG is an abbreviation for delphinidin, 3-pentose glycoside; D.di for delphinidin diglycoside; Cy. etc., for cyanidin derivatives, and P. etc., for pelargonidin derivatives.) Data chiefly from Hall (1940) and Beale, Price and Sturges (1941).

THE FATTY ACIDS OF THE FLACOURTIACEAE

Skin diseases are common and widely spread within the tropics and it is not surprising that inhabitants of those regions have turned to plants—and particularly to the fatty oils of plants—for the means to alleviate or to cure them. While many “native” remedies owe more to faith than to any real action against bacteria there are scores of such remedies which have proved their worth: among these we may reckon the oils of the Flacourtiaceae which have long been used in India (*Taraktogenos Kurzii* King), China (*Hydnocarpus anthelmintica* Pierre), Brazil (*Carpotroche brasiliensis* Endl.) and tropical Africa (*Oncoba echinata* Oliver).

The recognition of the real worth of these oils and of the peculiar acids which they contain is too long a story to repeat in detail here. The reader is referred to the chapter on “The Tree of the Leper” in Peattie’s *Cargoes and Harvests* (1932) and to the paper by Rock, Fairchild and Power (1922). We know today that all, or almost all, the plants whose oils have been shown to be effective in the treatment of leprosy contain glycerides of fatty acids of the Chaulmoogric acid series. These fatty acids are peculiar in that they have five-carbon rings. Starting with chaulmoogric acid (Power and Gornall, 1904) the series has been extended by the discovery of hydnocarpic acid (Power and Barrowcliff, 1905a; Barrowcliff and Power, 1907; Shriner and Adams, 1925), of gorlic or dehydrochaulmoogric acid (André and Jouatte, 1928; Paget, 1937; Cole and Cardoso, 1938a), of ketochaulmoogric acid and of ketohydnocarpic acid (Paget, 1937), and of a whole series of lower homologues of chaulmoogric acid, named aleptic, alepylic, aleprestic and aleprolic acids by Cole and Cardoso (1939a). These are listed with their formulae and some properties in Table II.

These acids, having an asymmetric carbon-atom in the ring (Fig. 3), are all optically active and strongly dextro-rotatory. This is a very useful property since the fats and oils of plants which do not have acids of the chaulmoogric series are almost always optically inactive (but see below). Hence any fat or fatty oil (not essential oil) which is markedly dextro-rotatory is almost certain to contain acids of this series.

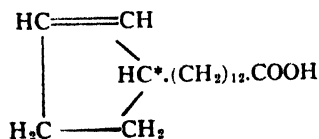


FIGURE 3.—Chaulmoogric acid (the C atom marked * is asymmetric).

TABLE II
FATTY ACIDS OF THE CHAULMOOGRIC ACID SERIES

Name of Acid	Formula	$[\alpha]_D^{25}$	M.Pt°C
Aleprolic.....	$C_8H_7.COOH$	+120.5 (Calc.'d)	
?	$C_8H_7.(CH_2)_2.COOH$	+110.5 (")	
Aleprestic.....	$C_8H_7.(CH_2)_4.COOH$	+100.5 (")	
Alepylic.....	$C_8H_7.(CH_2)_6.COOH$	+ 90.8	32
Alepic.....	$C_8H_7.(CH_2)_8.COOH$	+ 77.1	48
Hydnocarpic....	$C_8H_7.(CH_2)_{10}.COOH$	+ 68.0	58
Chaulmoogric...	$C_8H_7.(CH_2)_{12}.COOH$	+ 62.0	68
Dehydrochaulmoogric(Gorlic)	$C_8H_7.(CH_2)_8CH:CH(CH_2)_4.COOH$	+ 50.1	6
Ketohydnocarpic	$C_8H_6O.(CH_2)_{10}.COOH$		
Ketochaulmoogric.....	$C_8H_6O.(CH_2)_{12}.COOH$		

It was early realized that these acids are found only in plants belonging to a single tropical family—the Flacourtiaceae. Stranger yet, they occur only in two tribes of the family, if we accept the classification laid down by Gilg (1925), and given in outline in Fig. 4.

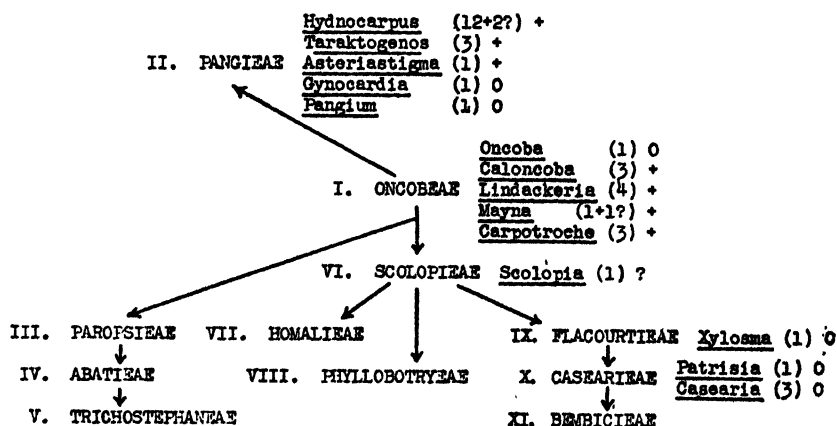


FIGURE 4 — Flacourtiaceae as sub-divided by Gilg (1925), to show distribution of genera containing optically active fatty acids (+). Numbers of species investigated are indicated by brackets. O indicates that acids are inactive. The sections of *Hydnocarpus* have been treated as separate genera—*Hydnocarpus*, *Taraktogenos* and *Asteriastigma*.

This seems to have been realized as long ago as 1926 by de Pupo: "Na apreciação dos schemas de E. Gilg encontramos a expressão botânica das verificações de Golding, Akers, Antenor Machado e Carneiro Felipe, nas íntimas relações das Tribus Oncobae e Pangicae, às quaes se filiam os generos *Hydnocarpus*, *Caloncoba*, *Carpotroche*, *Lindackeria* e *Mayna*." Kuhlmann, a little later (1929), monographing the Brazilian species of the Flacourtiaceae which are antileprotic, says: "In these notes, we are dealing with the Oncobae Tribe alone, because, in Brazil, only the species affiliated to it contain in their seeds an oil analogous to that of chaulmoogra, that is optically active."

The statements that the chaulmoogric series of fatty acids occurs only in the Flacourtiaceae and within that family only in the Oncobae and Pangicae are supported by a great amount of evidence, though more yet is desirable. In Table III we have listed the Flacourtiaceous plants whose chemistry has been studied, noting optical activity of the seed-fats or oils, the active and non-active acids which occur, and finally the presence or absence of cyanogenetic glycosides.

It will be seen that in the tribe Oncobae, regarded by Gilg (1925) as the plexus from which the other ten tribes have been derived, four of the five genera have optically active acids and none have been found to contain cyanogenetic glycosides. The absence of active acids from *Oncoba* is very interesting. The genus included the plants now called *Caloncoba* until Gilg (without a knowledge of chemistry) split the genus into two. Peirier (1929) writes appreciatively of Gilg's acumen in this respect: "... il tendrait à laisser penser que Gilg était heureusement inspiré lorsque, d'après les seuls caractères botaniques — (et notamment la forme coudée ou non de la partie supérieure du pétiole)—il démembrait le genre *Oncoba*, en laissant dans ce genre primitif quelques espèces telles que l'*Oncoba spinosa* et plaçant la plupart des autres, tels que l'*O. echinata*, *glauca*, *Welwitschii* dans le nouveau genre *Caloncoba*."

It is encouraging to find the facts of chemistry supporting a botanical split such as this, but one wonders if the genus *Oncoba* with no active acids is really primitive. It seems more likely that the power to form acids of the chaulmoogric series is primitive in the Flacourtiaceae and that it has been lost in those members of the family which do not have them today. *Oncoba*, in this respect, would be less primitive than *Caloncoba*.

The genus *Carpotroche* has been rather well investigated and the chemistry of *C. brasiliensis* (Raddi) Endl. is about as well known as that of any Flacourtiaceous plant. Its oil contains large amounts of hydnocarpic, chaulmoogric and dehydrochaulmoogric acids, as well as

TABLE III—FLACOURTIACEAE

Tribe	I: <u>ONCOCARPEAE</u>												
Genera and Species	<u>Oncoba spinosa</u> Forsk.	<u>Caloncoba glauca</u> (Beauv.) Gilg. <u>C. Melwitschii</u> (Oliv.) Gilg. <u>C. echinata</u> (Oliv.) Gilg. (see note)			<u>Lindackeria latifolia</u> Benth. <u>L. maynensis</u> Poepp. & Endl <u>L. rauciflora</u> Benth. <u>L. paraensis</u> Kuhlmann				<u>Mayna odorata</u> Aubl. <u>M. echinata</u> Spruce (see note)		<u>Carpotroche longifolia</u> (Poepp. & Endl.) Benth. <u>C. brasiliensis</u> (Raddi) Endl. <u>C. integrifolia</u> Kuhlmann		
1. Fat or oil	0	+33	+37	+52	+41	+46	+39	+43	+50	+50	+41	+54	+25
2. Aleprolic				?								?	
3. Aleprestic				?								?	
4. Alepylic				?								?	
5. Alepic				?								?	
6. Hydnocarpic	0		0?	0								45	
7. Chaulmoogric	0		+	75								24	
8. Dehydrochaulmoogric				15								15	
9. Ketohydnocarpic													
10. Ketchaulmoogric				Andre;									
11. Palmitic				8								7	
12. Stearic													
13. Arachidic													
14. Oleic				2								6	
15. Isogadoleic													
16. Linoleic													
17. Linolenic													
18. Cyanogenetic glycosides													
	Cole('35); Anon.('25)	Peirier('29)	Peirier('29); Cole('33)	Gould & A. Cole & C. etc.	Kuhlmann('28-9)	Kuhlmann('28-9)	Pupo('26)	Kuhlmann('28-9)	Pupo('26)	Kuhlmann('28-9)	Pupo('26)	see text	Kuhlmann('28-9)

In most cases the latest available figure is given. Specific rotation in degrees, other figures as percentage.

TABLE III—Continued

Tribe	II: <u>PANGIACEAE</u>														
Genera and Species	<u>Hydnocarpus venenata</u> Gardn. <u>H. Wightiana</u> Blume (see text) <u>H. alpina</u> Wight. <u>H. castanea</u> Hook. f. & Thoms. <u>H. anthelmintica</u> Pierre <u>H. subfalcata</u> Merr. <u>H. alcala</u> C. DC. <u>H. Hutchinsonii</u> Merr. <u>H. ovoides</u> Elm. (see text) <u>H. cauliflora</u> Merr. (see note) <u>H. octandra</u> Thw. <u>H. damensis</u> Park & Fisher <u>H. setumpall</u> v. Slooten <u>H. verrucosa</u> Park & Fisher <u>H. Woodii</u> Merr.														
1. $[a]_D$ fat or oil	+46	+51	+48	?	+50	+49	+48	+44	+1*	+42	+54	+39		+44	+46
2. Aleprolic		}			}										
3. Aleprestic		}			}										
4. Alepylic		}			}										
5. Alepic		}			}										
6. Hydnocarpic	+	49	0?		6d	+	0?	+			+	?	0?	+?	+
7. Chaumoogric	+	27	+		9	+	90	+			+	+	+	+?	+
8. Dehydrochaumoogric		12			1										
9. Ketohydnocarpic	Possolo ('39)														
10. Ketochaulmoogric	Andre;														
11. Palmitic			2		7		10								
12. Stearic								+							
13. Arachidic															
14. Oleic			6		10		+								
15. Isogadoleic	Cole ('33)														
16. Linoleic		Henry													
17. Linolenic			Anon. ('26);												
18. Cyanogenetic glycosides	Brill ('16); Park. & C. ('23) Cole & C. ('39) Andre; Cole ('33) Georgi & T.; Cole & C. ('39) Parkins & C. ('23); Cole ('33); Possolo ('39) Brill ('17); Park. & C. Possolo ('39) Park. & Cruz; Klingmiller; Possolo Park., C. & Reyes ('27) Park., C. & R. ('27); Cole ('33) Anon. ('26); Cole ('33) Aiyar et al. ('30); Cole ('33) Koolhaas ('30) Aiyar et al. ('30) Park. & C. ('23); Cole ('33); Possolo ('39); Anon ('29)														

* $[a]_D$ of fatty acids in the case of *H. ovoides*.

TABLE III—Continued

Tribe	<u>PANGIINAE</u> (Cont'd)						VI	IX	X			
Genera and Species	<u>Tarakoneus</u> <u>Kursi</u> <u>King</u>	<u>T. ilicifolia</u> <u>King</u> (see text)	<u>T. heterophylla</u> <u>Bl.</u> (see text)	<u>Asteriantigum</u> <u>macrocarpum</u> <u>Bedd.</u>	<u>Gynocardia odorata</u> <u>R. Br.</u>	<u>Pangium edule</u> <u>Rehm.</u>	<u>Scolecopia crenata</u> <u>Clos</u>	<u>Xylocopa Zalmanni</u> <u>Kiehl.</u>	<u>Patrisia</u> <u>Sp.</u>	<u>Cassaria pauciflora</u> <u>Comb.</u>	<u>C. singularis</u> <u>Kiehl.</u>	<u>C. Sp.</u>
1. [M] _D fat or oil	+50	+51	+43	+48	0	+9*	?	0	0	0	0	0
2. Aleprolic	}	<1		Alyar et al. Posolo ('37)	0	0						
3. Aleprestic												
4. Alepylic												
5. Alepic												
6. Hydnocarpic	35	+	+	?	0	0						
7. Chaulmoogric	22	+	+	+	0	0						
8. Dehydrochaulmoogric	23											
9. Ketchydnocarpic	Cole ('33)			Park. et al. (127)	Park. & C. (123)							
10. Ketchaulmoogric												
11. Palmitic	4			?								
12. Stearic					+	+						
13. Arachidic	?					+						
14. Oleic	15			7	+	+						
15. Isogadoleic	+											
16. Linoleic					+							
17. Linolenic					+							
18. Cyanogenetic glycosides	+			+	+	+						
	Basim ('26)	Marcen ('26); Cole ('33)	Ecolbas ('30)	Peacock & F. (131)	Power & B. (109b);	Brill ('17) Park. & C. ('23)	Kafuku, H., & Fuj. ('34)	Kohlmann ('26-9)	Kohlmann ('26-9)	Kohlmann ('26-9)	Kohlmann ('26-9)	Kohlmann ('26-9)

* [a]_D of fatty acids in the case of *Pangium edule*.

smaller amounts of ketohydnocarpic and ketochaulmoogric acids (Da Silva, 1926; Paget, 1937; Cole and Cardoso, 1938a and b). It may also have the lower homologues of chaulmoogric acid.

Turning to the Pangieae, which Gilg places next to the Oncobeeae, we find that the large genus *Hydnocarpus* has many spp. with chaulmoogric acid. Warburg (1895) merged *Asteriastigma* and *Taraktogenos* with *Hydnocarpus* and was followed in this by Gilg (1925) and by Sleumer (1938). The known chemistry of the plants is not against this, but Fischer (1927) finds the flowers sufficiently distinct to justify the retention of separate genera—which we have done in Table III. According to Sleumer the correct name for *H. Wightiana* is *H. laurifolia* (Dennstedt) Sleumer, but we have retained the more familiar name.

The remaining genera *Gynocardia* and *Pangium* do not contain acids of the chaulmoogric acid series. They do, however, have cyanogenetic glycosides, a character shared with *Taraktogenos*, *Asteriastigma* (probably), and *Hydnocarpus* (Cole, 1933; Grimme, 1911?). So far as we know, these glycosides occur in the Flacourtiaceae only in the tribe Pangieae.

Information from the remaining nine tribes of the family is scarce. *Scolopia crenata* Clos of the Scolopieae has been investigated by Kafuku, Hata and Fujikawa (1934). Their paper contains no reference to optical activity. *Xylosma Zalmanii* Eichl. in the Flacourtieae has no active acids, while one species of *Patrisia* (*Ryania*) and three of *Casearia* in the Casearieae are also lacking in acids of the chaulmoogric series. It is to be hoped that this list will soon be greatly extended.

So far in this section we have considered only the Flacourtiaceae. How certain are we that the peculiar acids of this family do not occur elsewhere? We cannot, of course, be completely certain until *all* plants have been examined but enough is known now to make it seem unlikely that these acids will be found elsewhere. In many cases the optical inactivity of fats or oils is not stated but if analyses indicating about 100 per cent non-active acids are available that is just as good. Accordingly, in Table IV we have listed analyses of plants belonging to several orders, but more particularly those belonging to the same order as the Flacourtiaceae (Parietales of Engler and Prantl) and to neighbouring orders. This list, extensive as it is, is by no means complete, but it does give some idea of the situation.

In no case is the seed-fat or oil strongly dextro-rotatory. Some members of the Euphorbiaceae yield oils with small optical activity, *Ricinus communis* L. being noteworthy in this respect. Rotation here is at least partly due to large amounts of ricinoleic acid ($[\alpha]_D + 6.67^\circ$)

TABLE IV

Order	RHOBO.	GERANIALES			
Family	<u>CAP.</u>	<u>SIMARUBACEAE</u>		<u>BURSER.</u>	<u>MELIACEAE</u>
Genera and Species	<u>Crateva Benthemii</u> <u>Kiehl.</u>	<u>Picramnia quassaloides</u> <u>Benth.</u> <u>Alseodaphne glandulosa</u> <u>Desf.</u> <u>Irvingia gabonensis</u> <u>Beill. (see note)</u> <u>I. Oliveri</u> <u>Pierre</u> <u>I. Smithii</u> <u>Hook. f.</u> <u>Picramnia Camboita</u> <u>Engl. (see note)</u>		<u>Gomphopore sandibar-</u> <u>ica Engl.</u> <u>Canarium commune L.</u>	<u>Ascaros Rohituka</u> <u>W. & A.</u> <u>Cabralia laevis</u> <u>C.DC.</u> <u>Guarea trichiloides</u> <u>L.</u> <u>Melia indica</u> <u>Brandis</u>
1. [ω] _D fat or oil	0				0
2. capric		+			
3. lauric			39		
4. myristic			33		41
5. palmitic		+	10	30	15
6. stearic			2	10	
7. arachidic			1		
8. decenoic			3		
9. hexadecenoic					
10. oleic		++	80	40	11
11. petroselinic		+	2		
12. elaidic			5		
13. isogadoleic					
14. erucic					
15. linoleic		++			57 ^a
16. linolenic		++			
17. ricinoleic					
18. lignoceric					
19. tariric		0			
20. tiglic	Kuhlmann ('26-9)	Mik. (136)	Bontoux ('10)	Steger & van Loon ('33)	Kuhlmann ('26-9)
			Piersaerts ('22)		Kuhlmann ('26-9)
				Anon. ('26)	
				Steg. & van Loon ('140)	
				W. & Pat. ('35)	
					Child & R. ('36)
21. total fatty acids		X.	97 100 100	100	100
22. cyanogenetic glycosides		Tauji. (133)	Bush, M. (139)	0	0

^a includes linoleic and "isomeric linoleic."^b no chaulmoogric, no hydnocarpic.

TABLE IV—Continued

Order	GERANIALES (cont'd.)				SAPINDALES			
Family	<u>EUPHORBACEAE</u>				<u>BUX.</u>	<u>ANACARDIACEAE</u>		
Genera and Species	<u>Patranjiva Roxburghii</u> Wall. <u>Croton tiglium</u> L. <u>Aleurites moluccana</u> Willd. <u>Hevea brasiliensis</u> Muell. Arg. <u>Flukonetia tamnoides</u> Muell. Arg. <u>Ricinus communis</u> L. <u>Euphorbia Cyparissias</u> (see note) <u>E. marginata</u> Fursh. <u>Foranthera micro-</u> <u>phylla</u> Brongn.				<u>Simmondsia californica</u> Nutt.	<u>Ancardium occidentale</u> L. <u>Buchanania angustifolia</u> Roxb. <u>Pistacia lentiscus</u> L. <u>P. vera</u> L.		
1. [α] _D fat or oil		0	0	+5	+3			0
2. capric								
3. lauric	tr.							
4. myristic	7							
5. palmitic	0		6					
6. stearic	+ tr.		9	<1	+	17	tr. 29	1 25
7. arachidic	1	tr.					8	12 2
8. decenoic					0?			
9. hexadecenoic								
10. oleic	+ 37	26		7	tr.	60	57	46 70
11. petroselinic								
12. elaidic								
13. isogadoleic								
14. erucic								
15. linoleic	+ 19	Kuhlmann ('26-9)	30	4			5	6 20
16. linolenic		7amieson & B. ('30)	18					
17. ricinoleic		Kuhlmann ('26-9)		88				
18. lignoceric	+							
19. tauric								
20. tiglic	tr.							
21. total fatty acids		69		99+			97	99 91 101
22. cyanogenetic glycosides	Krich & P. ('31) Fleisch & Wolff ('34)			Andre ('25) c	+			

^aCyanogenetic glycosides occur in some spp. of *Euphorbia* (Finnemore & Cox, 1929).

TABLE IV—Continued

Order	SAPINDALES (Cont'd.)					
Family	<u>ANACAR.</u> (Cont'd.)	<u>CELASTR.</u>	<u>SALVAD.</u>	<u>STAPHYL.</u>	<u>ICAC.</u>	<u>SAPINDACEAE</u>
Genera and Species	<u>Mangifera indica</u> L.	<u>Celastrus paniculatus</u> Willd. <u>C. scandens</u> L.	<u>Salvadora oleoides</u> Dcne. (see text) <u>S. Persica</u> Garcin	<u>Staphylea pinnata</u> L. <u>Staphylea</u> sp.	<u>Sarcostigma Kleinii</u> W. & A. (see text) <u>Sapindus marginatus</u> Willd. <u>Schleichera oleosa</u> Kerr. <u>Neobellium leppaceum</u> L. <u>N. mutabile</u> Bl. <u>Parenepbellium</u> sp. <u>Ungnadia speciosa</u>	
1. [C ₁₇] fat or oil					*1	
2. capric			1			0
3. lauric			21			1
4. myristic			53			4
5. palmitic		20	19	4		6
6. stearic	+	8	19			2
7. arachidic		2			se ^d acids 3%	0?
8. decenoic						23
9. hexadecenoic						35
10. oleic	+	15	5	53	55	63
11. petroselinic			5	94		60
12. elaidic						45
13. isogadoleic						44
14. erucic						70
15. linoleic		39		38		15
16. linolenic		12		41	11	4
17. ricinoleic		38				
18. lignoceric		21				
19. tariric						
20. tiglic						
21. total fatty Acids	Amon. (120) Wilkins (142) Gunde & Hild. (156) Barkunbus & Kresson (132) Patel et al. Gunde & H. (133) Gunde & Hild. (133)	90 ^d 69 ^e	99 99	95 100	100	78+ 96 96 100
22. cyanogenetic glycosides	0					+

^d esters of formic, acetic and benzoic acids with a tetrahydroxylic compound also present to extent of 10%.

^e esters of formic, acetic and caproic acids present.

TABLE IV—Continued

Order	PARIETALES (Cont'd.)							PRIM.	TUBI.
Family	DIPTERO.	BIX.	COCH.	VIOL.	FL.	PASSIFLOR.	CAR- TO.	THP- ORH.	MART.
Genera and Species	<i>Shorea robusta</i> Gaertn. f. <i>Vateria indica</i> L.	<i>Bixa orellana</i> L. (see text)	<i>Cochlospermum</i> <i>orinocense</i> Steud.	<i>Leonia</i> sp.		<i>Passiflora ovata</i> Vell. <i>P. edulis</i> Sims <i>P. alliacosa</i> Barb. Rodr.	<i>Carica Papaya</i> L.	<i>Clavija macrophylla</i> Blq.	<i>Martynia diandra</i> Glox.
1. [- ¹] _D fat or oil		0	0	0	See Table I for Miscourtiaceae	0		0	
2. capric									
3. lauric									
4. myristic									
5. palmitic	4	10 ⁶	+			7	12		6-10
6. stearic	44	39	+			1	5		6-11
7. arachidic	6	3 ⁶							17
8. decanoic									
9. hexadecanoic									
10. oleic	42	46	+			20	50		36-74
11. petroselinic									
12. elaidic		+							
13. isogadoleic									
14. erucic									
15. linoleic	3					63	2		6-32
16. linolenic									
17. ricinoleic									
18. lignoceric									
19. tariric									
20. tiglic	Hild. & Z. ('42) Hild. & S. (131)	Aiyar ('22) Chaulmogrisc absent	Ehrlmann ('26-9)	Kuhlmann ('26-9)		Jameson & M. ('24) Kuhlmann ('26-9)	Loesche & H. ('37)	Kuhlmann ('26-9)	Royal & D. (139) Rags et al (144)
21. total fatty acids	99 100+					98+	99+		90-96
22. cyanogenetic glycosides	0					None ('32)			

⁶ absent, say Puntambekar and Krishna, 1933.

TABLE IV—Continued

Order	RUBI.	CUCURB.
Family	<u>RUBI.</u>	<u>CUCURB.</u>
Genera and Species	<u>Posoqueria lati- folia R. & Sch.</u>	<u>Fevillea trilobata</u> L. <u>Hodgsonia capnic- carpa Ridley</u>
1. $[\alpha]_D$ fat or oil	0	0
2. capric		
3. lauric		
4. myristic		41
5. palmitic		37
6. stearic		9
7. arachidic		41
8. decenoic		
9. hexadecenoic		41
10. oleic		27
11. petroselinic		
12. elaidic		
13. isogadoleic		
14. erucic		
15. linoleic		25
17. ricinoleic		
18. lignoceric		
19. tariric		
20. tiglic		
21. total fatty acids		Hild., M. & P. (139)
22. cyanogenetic glycosides		

which occurs as a glyceride ($[\alpha]_D + 3^\circ$) in castor oil. Gillot (1927a and b, 1928, 1933) examined several species of *Euphorbia* and found all the oils to be very slightly dextro-rotatory. The seed-oil of *Sarcostigma Kleinii* W. & A. (Icacinaceae) is also slightly active.

Analyses totalling 100 per cent or near it of non-active fatty acids have been recorded for members of the Simarubaceae, Burseraceae, Meliaceae, and Euphorbiaceae within the Geraniales; for Anacardiaceae, Celastraceae, Salvadoraceae, Staphyleaceae, Icacinaceae, and Sapindaceae of the Sapindales; and for Caryocaraceae, Theaceae, Guttiferae, Dipterocarpaceae, Passifloraceae, and Caricaceae of the Parietales. There are doubtless many other records for we have made no attempt to compile an exhaustive list.

Several points of interest are brought out in the Table. Within the Simarubaceae, lauric and myristic acids occur in very large amounts in *Irvingia* and may be characteristic of that genus. *Irvingia gabonensis* Baill. was at one time described as *Mangifera gabonensis* A. Lec. and included in the Anacardiaceae, but *Mangifera indica* has a fat composed almost entirely of oleodistearin with no or very little lauric or myristic acid. If this is true of other species of *Mangifera* we may conclude that *Irvingia* was rightly separated from the genus (though not necessarily from the family). It might be noted here that the fats of the Salvadoraceae also have much lauric and myristic acid. In the Simarubaceae, too, we have the curious tariric acid with its acetylenic linkage occurring in at least five species of *Picramnia* (Steger and van Loon, 1933). It is definitely absent from *Picrasma quasstoides* Benn. of the same family, and according to Hilditch (1940) is at present known only from *Picramnia*. This acid may characterize *Picramnia* just as the chaulmoogric acids characterize the Oncobaceae and Pangieae—but it should be searched for diligently before we jump to conclusions on this point.

The family Buxaceae provides in *Simmondsia californica* at least one case as remarkable as that of *Picramnia* and it is a pity that we have no further analyses. Greene and Foster (1933) found that the "seed-oil" of this plant is actually a liquid wax. This has been confirmed by McKinney and Jamieson (1936). The rather general distribution of alkaloids in the Buxaceae noted by Martin-Sans (1930) suggests that these, rather than fatty acids might better be used to characterize this family.

Some members of the Sapindaceae have large amounts of arachidic acid in their seed-fats. While this acid occurs in many other plants it is usually in small amount. It may not, however, be characteristic of the Sapindaceae as a whole for it is said by Dermer and Crews (1939)

to be absent or in very small amount in *Sapindus marginatus* Willd., though there is about 22 per cent in *S. trifolius* L. (Paranjpe and Ayyar 1929 ?).

We may now briefly consider the families of the Parietales—the more immediate neighbours of the Flacourtiaceae. There is nothing very remarkable about the fatty acids of these plants unless it be the high proportions of stearic + oleic acids found. Of the plants listed in the Guttiferae eight, belonging to five genera, have values of 78, 59, 96, 96, 97, 95 and 94 per cent respectively for stearic + oleic acids. In other families of the same sub-order (Theineae), we have 94 per cent in *Caryocar villosum* of the Caryocaraceae, 89 per cent in *Thea sinensis* (Theaceae) and 86 per cent and 87 per cent in two members of the Dipterocarpaceae.

In the sub-order Flacourtiineae, we have few analyses listed and it is not possible from the figures available to say if the family Flacourtiaceae is well placed. This was noted by Claus (1940).

THE CHEMISTRY OF THE CUPRESSACEAE

To deal fully with the chemistry of even a single family of the Coniferales would be an enormous task and well beyond the scope of this paper, for the conifers are provided with essential oils in wood, leaf and cone, essential oils which are exceedingly complex. The synonymity involved in the chemistry of these oils is as discouraging as that found in plant taxonomy! Almost every investigator has found a “new compound” and has provided a new name—in many cases without any really adequate characterization. We have listed in a preliminary check-up over one hundred “constituents” of the Cupressaceae, but for our present purposes this number has been much reduced.

In Table V we have listed the genera and some species of the family as arranged by Pilger (1926), some twenty constituents, and the response (+ or -) to the Mäule reaction—which, as we have shown, almost certainly means the presence or absence of the syringyl group in the lignin of the plant concerned (Creighton, Gibbs and Hibbert, 1944).

Lignins of plants belonging to the Coniferales almost always yield negative results with the Mäule reaction. The writer (in Creighton, Gibbs and Hibbert, 1944) found, however, that several species of *Podocarpus* give positive results, as does *Tetraclinis articulata*. Creighton (*loc. cit.*), using the same material, found that these plants give syringyl aldehyde when their lignins are subjected to alkaline nitrobenzene oxidation. We believe that a positive Mäule test indicates the pres-

ence of the syringyl group. It will be seen from Table V that *Tetraclinis articulata* alone of the Cupressaceae gives a positive Mäule reaction. This is of considerable interest in view of the taxonomic history of this plant. Although native to the western end of the Mediterranean it was formerly described as *Thuja articulata* Vahl, as *Callitris quadrivalvis* Vent., and as *C. articulata* (Vahl) Murbeck. *Thuja* is widely spread in east Asia and North America; *Callitris* is confined (if that is the word) to Australia, Tasmania and New Caledonia. Masters (1895) first established the genus *Tetraclinis* with the sole species *Tetraclinis articulata* (Vahl) Masters, and this separation from *Callitris* and *Thuja* would certainly seem to be justified chemically (Table V). Hayata (1932) would even make a separate family, the Tetraclinaceae.

Early workers included *Widdringtonia* with *Callitris* and Saxton more recently (1934) says that *Callitris*, *Widdringtonia*, and a third genus, *Actinostrobus*, all agree in their early embryology. The last genus (or, rather, one of its two species) resembles *Callitris* in having manganese compounds in its wood and geranyl acetate in its essential oil: we have all too little information about *Widdringtonia*. Incidentally, the "callitrol" of Baker and Smith (1910) has been shown by Trikojus and White (1932) to be 1-citronellic acid.

We shall pass over the other genera of the family, but an inspection of Table V will show that the chemistry supports in some degree the taxonomic arrangement. A more detailed discussion of these plants is planned for the future.

TABLE V—CUPRESSACEAE

	<u>Actinostrobus</u> <u>Pyramidalis</u> Mq.	<u>Callitris Macleayana</u> <u>F. v. M.</u> <u>C. robusta</u> R. Br. <u>C. glauca</u> R. Br. <u>C. rhomboidea</u> R. Br. <u>C. calcarata</u> R. Br.	<u>Tetradlepis articulata</u> <u>(Vahl)</u> Masters	<u>Callitropis araucarioides</u> Comp.	<u>Middingtonia juniperoides</u> (L.) Endl. <u>W. cupressoides</u> (L.) Endl. <u>W. Schwarzii</u> (Merl.) Masters	<u>Pittroya cupressoides</u>
1. Maule reaction ¹	G ⁺	G ⁺ ² G ⁻ G ⁻ G ⁻ G ⁻	G ⁺		G ⁺ ³ G ⁻ G ⁻	G ⁻
2. Mn compounds	+	+	+	+	+	+
3. Guaijol		+	+			
4. l-Citronelllic acid		+	+	+	0 (one sp.)	
5. Geranyl acetate	+	+	+	+	+	
6. Carvacrol				+		
7. Thymohydroquinone			+	+		
8. Thymoquinone			+	+		
9. Thujyl alcohol						
10. Thujone						
1. Sabinene						
2. Sabinol						
3. Cedrol						
4. Shonanic acid						
5. Sylvestrene						
6. p-Cymene						
7. Phellandrene						
8. α-Terpinene						
9. γ-Terpinene						
10. δ-Cadinene						
21. l-Cadinene						

¹C (Crocker, 1935), E (Evans, MSS.), G (Gibbs, unpublished), S (Schindler, 1931).

TABLE V—Continued

	<u>Disela Archeri</u> J.D. Hook	<u>Thuja dolabrata</u> (L.f.) S. & Z.	<u>Thuja occidentalis</u> L. <u>T. plicata</u> Don. <u>T. orientalis</u> L. (Biota o.)	<u>Libocedrus decurrens</u> Torr.	<u>L. macrolepis</u> (Kurz) Benth.	<u>L. formosana</u> Florin	<u>Fokienia hodginsii</u> (Gunn) H. & T.	<u>Cupressus sempervirens</u> L.	<u>C. macrocarpa</u> Gord.	<u>C. Goveiana</u> Gord.	<u>C. lusitanica</u> Mill.	<u>C. torulosa</u> Don.
1. Mule reaction	O ^a	O ^a	O ^a	O ^a	O ^a		O ^a	O ^a	O ^a	O ^a		
2. Mn compounds												
3. Guaiol												
4. l-citronellollic acid												
5. Geranyl acetate												
6. Carvacrol												
7. Thymoquinone												
8. Thymoquinone												
9. Thujyl alcohol												
10. Thujone												
11. Sabinene												
12. Sabinol												
13. Cedrol												
14. Shonanilic acid												
15. Sylvestrene												
16. p-Cymene												
17. Phellandrene												
18. α-Terpinene												
19. γ-Terpinene												
20. d-Cadinene												
21. l-Cadinene												

^aabsent in galbanum. ^babsent in galbanum, present in leaf-oil. ^cpercentage.

TABLE V—Continued

	<u>Chamaecyparis obtusa</u> S. & Z.	<u>C. forbesensis</u> Matsum.	<u>C. Lawsoniana</u> (Andr.) Parl.	<u>C. thuyoides</u> (L.) B.S.P.	<u>C. nootkatensis</u> (Lamb.) C. obtusa S. & Z. f. formosana Hay.	<u>Arceuthobium drupacea</u> (Lab.) A. & K.	<u>Juniperus communis</u> L.	<u>J. rigida</u> S. & Z.	<u>J. oxycedrus</u> L.	<u>J. Setina</u> L.	<u>J. excelsa</u> Marsch. Bieb.	<u>J. chinensis</u> L.	<u>J. phoenicea</u> L.	<u>J. barbadensis</u> L.	<u>J. occidentalis</u> Hook.	<u>J. virginiana</u> L.
1. Mäule reaction	Q ^a	S ^a	Q ^a	Q ^a			Q ^a	Q ^a				Q ^a	W ^a	Q ^a		
2. Mn compounds																
3. Guaijol																
4. 1-Citronellol		?														
5. Geranyl acetate																
6. Carvacrol																
7. Thymohydroquinone																
8. Thymoquinone																
9. Thujyl alcohol																
10. Thujone																
11. Sabinene																
12. Sabinol																
13. Cedrol																
14. Shonanilic acid																
15. Sylvestrene																
16. p-Cymene																
17. Phellandrene																
18. α-Terpinene																
19. γ-Terpinene																
20. d-Cadinene																
21. l-Cadinene																

percentage. ^a in bark, but absent from leaves.

CONCLUSIONS

We have attempted, in this brief paper, to illustrate the thesis that comparative chemistry is an important tool in taxonomy—a tool that will improve with use. We need much more information, however, from non-economic as well as from economically important plants, and this should be *selective*, the plants examined being those whose chemistry is most likely to contribute to the solution of taxonomic problems.

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TERMINAL PULMONARY VENULES IN MAMMALIAN LUNGS

By CHARLES C. MACKLIN, F.R.S.C.

HISTOLOGY shows that venules and other parts of the pulmonary vascular system are so intimately built into the lung tissue around them that, in normal young human subjects and animals, particularly during vigorous exercise, they are lengthened and widened in inspiration, and shortened and narrowed in expiration. Thus, by implication, the pulmonary blood volume is successively increased and diminished in each respiratory cycle, inspiration involving what might be called a diastole of the pulmonary vessels, and expiration a systole. In the hope that this conception would shed new light on the lesser circulation, microsections of expanded lung from eleven species of mammal were studied and implications drawn.

These mammals are man, domestic cat, rabbit, monkey, guinea-pig, goat, dog, baboon, rat, mouse and pig. Fixation, with Bouin's fluid (Masson, 1929), was usually by perfusion of the pulmonary vessels in the unopened thorax (Hartroft and Macklin, 1944a), but some lungs were filled by tracheal injection (Hartroft and Macklin, 1943a), and the pig material was immersed. Further details are included with the presentation of histological evidence and description of plates. Blocks were routinely taken from each of the seven lobes or corresponding regions, so that the various parts of the lungs were thoroughly scanned. Standard techniques for dehydration, embedding in paraffin, sectioning and staining were used. Over 3700 stained sections were available, representing sixty-four pairs of lungs.

HISTOLOGICAL EVIDENCE

An expansile sleeve of air sacs is to be seen around each of the pulmonary venules and arterioles in the twenty-seven photomicrographs on the five plates. These small vessels are, in respect of their environment, representative of hundreds to be seen in this micro-section *cache*. The same sort of distensible investment might have been photographed for the many larger branches of the two pulmonary vascular trees. The illustrations may now be examined systematically.

PLATE I

Human lung.

This section was cut from the left upper lobe of a white woman of thirty-one years. The fresh, slightly oedematous lungs were fixed

intrabronchially, the degree of distention being controlled by measurements of the pleural cavities. Above is seen a venule v . Affixed to the vessel wall are the bases of surrounding alveoli of the "marginal" type (Macklin, 1944, 1945). Some of these alveoli have been so cut as to present open outlines a , revealing the alveolar space as continuous with that of the main cavity of the alveolar sac to which it belongs, while others show closed outlines a_1 resulting from a tangential cut which has not included the alveolar mouth, and so the enclosed space of the alveolus does not present any confluence with that of its sac

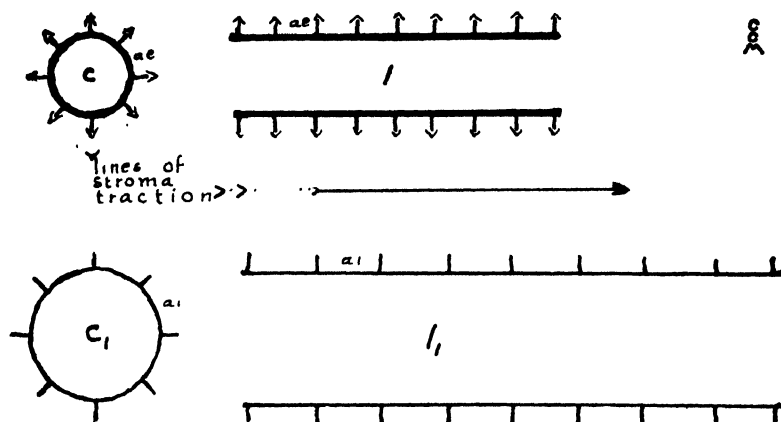


FIGURE 1.—A diagram to show the expansile and extensile action on a pulmonary blood vessel of inflation of the surrounding alveoli. c and l show the same vessel in cross and longitudinal section in the phase of *expiration*; and c_1 and l_1 give the same views of the same vessel in the phase of *inspiration*. ae is an alveolus in expiration, and a_1 is the same in inspiration. Only the bases and part of the side walls of the alveoli are seen.

(Hartroft and Macklin, 1944a). Collectively these perivascular alveolar bases form a tunnel enclosing the vessel (Fig. 1), and adhering to it. This tunnel is altered in length and width when the walls of these air sacs stretch and recoil in ventilational action, because it is composed of parts of the actual walls of these sacs, and is connected by means of the lung stroma with other parts of the walls of these and adjoining air sacs. The walls of the air sacs are made up largely of fibrous tissue. The fibers are of two types, white and yellow, the former being inextensible, like tendon material, and the latter elastic. Stretching of the air sac walls is attended by a radial pull upon the vascular walls, and also by a longitudinal pull (Fig. 1), just as with the walls of the air tract—the bronchi, bronchioli and alveolar ducts.

Deflation of the air spaces is accompanied by a recoil of the elastic tissue, and these tubes are all shortened and narrowed.

The immediate motivation in the enlargement of the air spaces is the pressure of the gas within them occasioned by the enlargement of the pleural cavity in inspiration, and the ultimate motivation is, of course, the action of the musculature of the thoracic walls and diaphragm (Best and Taylor, 1943). It is fortunate that the stroma is attached thus to the blood vessels, for otherwise there would not be this instant and favourable adjustment of their walls and caliber to the needs of the inspiratory phase. The inspiratory action which brings air into the lungs also brings in more blood. Stroma pull enlarges blood and air tracts during this phase. Similarly, pressure within the lymph vessels is then lowered and diffusion into them favoured. In expiration, with diminution of the size of the air sacs, there is a relaxation of tension on the walls of these vessels, and the recoil of their elastic elements returns them to a state as at the end of expiration.

There is thus, through stroma pull, in inspiration, a filling action, particularly on the blood vessels, but also upon the lymph vessels; and, through elastic recoil in expiration, a squeezing or milking action, tending to move the contents, blood and lymph, toward the hilus. The analogy of the pump is striking.

In the figure of human lung we see, below, a small vein v_2 , cut somewhat slantingly. The projection above and to the left is due to the conjunction of a tributary. As with the venule, the encircling alveoli are attached to the wall and would, by their rhythmic enlargement and diminution in respiratory movements, lead to synchronous and similar changes. This larger vessel is surrounded by a greater number of alveolar bases, and the underlying sheath of connective tissue is thicker than that of the venule. One can imagine how enlargement of the perivascular air spaces would mean an increase in the collective area of these bases, and this increase would involve an elongation and widening of the vein, assuming that no kinking occurred, and there is no histological evidence of this. Conversely, contraction of the perivascular air spaces would mean coincidental shortening and narrowing of the vein. Fig. 1, showing cross, c , c_1 , and longitudinal l , l_1 sections of a pulmonary blood vessel with its investment of alveoli in the contrasted states of expiration and inspiration, should make these morphological changes clear. That this areal increase and decrease in the bases is but a part of the general mural change in the air spaces of the vascular environment in passing from expiration to inspiration and *vice versa* should be realized from what has been said

as to the elongational and centrifugal action of stroma traction on the blood vessels.

A lymphatic vessel, *lym*, cut longitudinally, is seen on the left of these veins. Its thin wall bears the same relation to the contiguous alveolar bases as do those of the vein and venule, and hence inflation and deflation of these air spaces, in the light of what has been said, may be regarded as having a like action on it.

Cat lung.

The two cat lungs from which the four sections were selected were fixed in the unopened thorax by perfusion *via* the aorta, so preserving the state of inflation. In A a vein v_2 , empty of blood, is seen, and entering it a venule v , cut lengthwise, containing blood. Around both are alveolar sacs, *as*, the bases of whose marginal alveoli are adherent to the walls of these blood vessels. This is an excellent example of the way in which the distensible web of airholding tissue invests the pulmonary blood vessels so as to subject them to respiratory length and width adaptations.

B shows a cross-section of a pulmonary vein v_2 , partly filled with blood, surrounded by distended alveoli, whose bases are applied to the wall so closely as to constitute a part of it. Such a vein would participate in the inflational and deflational movements of its environmental associates.

C presents a very slanting section of a vein v_2 , with a venule v above, just about to enter it. The surrounding expansile spongework is clearly evident, and the alterational influence of its functional movement on such vessels is easy to visualize. These vessels happen to be not far from the pleura, but those in the depths of the lung are similarly influenced in dimensions by the respiratory movements of their immediate environs.

In D a fortunate cut has laid bare in longitudinal section a rivulet system of venules v entering a small vein v_2 . One can readily objectivize the various parts of such a system as they lengthen in inflation of the surrounding air sacs. Such lengthening, however, is not like that of a rubber tube when pulled upon, for, if it were merely that, there would be a coincidental narrowing in inspiration, with impediment to the flow of blood within; and we know that such an impediment does not occur. Fortunately, at this time, the stroma pulls upon the walls, widening them. So, in inspiration, the caliber of the lengthened vessel may not only be maintained, but actually increased.

PLATE II

Rabbit lung.

The photomicrographs are from five sections representing the upper, middle and lower lobes of two rabbits fixed *in situ* by vascular perfusion *via* the aorta. In A, B and C longitudinally cut venules v , v_1 appear entering larger ones v_2 seen in cross-section. In D a cross-section of a venule v is seen with four alveolar sacs as around it. E shows a somewhat larger vein v_2 with stumps of two tributaries below. In all cases the venules course between alveolar sacs. In life these vessels are enmeshed in tissue acting like a bellows in perpetual operation, and the movements of this bellows are transmitted to the vessels. In B, C and E, from the same rabbit, the venules contain blood and there has been some separation of plasma and corpuscles. Although these vessels were apparently not traversed by the Bouin's fluid, the fixation in this region is thorough, for the tissue was quite stiff when it was cut. In A and D, from another rabbit, the blood has been washed from the venules of the region. The particular veins seen in B, C and E, though not exposed to the internal pressure of the wash fluid and fixing fluid, are nevertheless open to about the same degree as those of A and D. The investment of expansile tissue is so firmly affixed to these venules that its expansion and contraction must produce in them changes in length and width (Fig. 1). In elongation the direction of shift is toward the source, that is, away from the wider end of the vessel. The venule of C is surrounded by alveolar sacs as , with their small, cuplike alveoli applied so closely to its walls that they are really a part of it. The applied parts of these marginal alveoli, the bases or floors, increase in area in inspiration as their alveolar sacs are enlarged, and similarly diminish in expiration, just as do those applied to the pleura. Since alveolar bases, side by side, forming a layer or membrane, make up the boundary of any alveolar sac, it is clear that the sac cannot actually enlarge without an areal increase of these bases. It is clear also that the collective effect of the simultaneous enlargement of the bases forming the outer adventitia of such venules would be not only to lengthen the vessels but also to widen them, unless there be wrinkling of the bases, and this is probably not the case in the living animal, although in fixed and dehydrated material there may be some distortion of this kind. It is therefore obvious that all such venules as v , and their number is legion, must undergo lengthening in inspiration and shortening in expiration in proportion to the degree of enlargement and contraction of their investing air sacs. The same changes occur in the larger venules, as v_1

and v_2 of C and the other figures. The relation is well seen in the bloodless venules of A. It is impossible for the alveolar sacs to be distended without influencing the length and width of the venules to which they are affixed; and since no sac is without affixed venules, and arterioles also, no sac can be without influence of this kind.

The relation of a small venule to its surrounding well-distended alveolar sacs is seen in cross-section in D. A somewhat longer stretch of a larger venule v_2 , split lengthwise, is seen in E, the expansion of whose investment of alveolar sacs would be attended by a pulling out in length and width. That such a venule could remain "frozen" in dimensions while its surroundings are enlarging and contracting is impossible to conceive. The lighter streaks in the interior are artificial cracks in the blood mass.

Among the pulmonary blood vessels the venules are not unique in being exposed to length and width modification by their immediately environing tissue, for all the intrapulmonic veins are influenced by the respiratory tides in the same way. True, the larger ones develop a thicker sheath, as seen in v_2 human, and v_2 monkey B, but because this is enveloped by expanding and contracting tissue they will extend and widen in inspiration, and contract and narrow in expiration. They are really a part of the lung stroma, and the rhythmic expansile pull and retraction of this involves them in these movements. The intrapulmonic arteries behave similarly, and sections show them, as we shall see, invested by the same sort of expansile sleeve as the veins. They, like the veins, are pulled longer and wider in inspiration by the action of the encompassing connective tissue stroma; and, as in the veins, the contraction of their elastic tissue fibers shortens and narrows them in expiration.

We may ask ourselves, what happens to the smooth muscle in the walls of these vessels during these form changes. Is there an automatic relaxation in inspiration and contraction in expiration? Some such adjustment has been postulated for the bronchial musculature, which is exposed to the same rhythmic changes (Macklin, 1929). The answer to this and many allied questions will have to await further enquiry. This paper is primarily concerned with the pulmonary venules, and they contain but little smooth muscle, and none at all as they pass over into the capillaries. In the attack upon the wider problem of involvement of the two vascular trees in pulmonic respiratory movements a thorough study should be made of the distribution of muscle throughout their walls in these various mammalian species. This wider problem should be studied in association with the allied problem of involvement of the bronchial tree in the pulmonic respir-

atory movements, for the air and blood tracts are bound up in the same stroma and are acted upon synchronously and in the same general way. The extensional and dilatational influence of the expanding alveolar sacs around them is felt by the air tract within the lungs throughout its extent, for this tract is enveloped in air sacs in a manner like that of the pulmonary arteries and veins. Inspiratory lengthening with widening, and expiratory shortening with narrowing, in all parts of the bronchial tree, have already been discoursed upon (Macklin, 1929). Relative to caliber, the range of these movements is greatest in the finer, peripheral parts of the air tract system, as it must also be in the blood tract system. It has been said that, with a rigid bronchial tree, inflation and deflation of the lung would be impossible. We may say, just as truly, that these respiratory movements of the lung would be quite as impossible if the arteries or veins were suddenly to become rigid. In either of these contingencies the lung would be firmly locked, except for the subpleural layer of alveolar sacs, and aeration of these would be greatly impeded. It is fortunate that pulmonary arteriosclerosis is not as serious nor obvious as it is in the systemic arterial tree (Macklin and Macklin, 1942), for otherwise grave interference with ventilation would ensue.

It is important to realize that in all parts of these conduits for air and blood there is normally a widening with elongation, or at least a maintenance of the caliber. This is hard for many people to comprehend, for they have in mind the unapt simile of the narrowing of a rubber tube as it is stretched; but, when they reflect that if this sort of action occurred there would be a retardation of flow of both air and blood, they have to rule it out, for such a retardation is fortunately not found in the normally functioning bodies of healthy young human subjects and other mammals. Such a contemplation of the caliber of air and blood tubes in action in the normal lung emphasizes the importance of centrifugal stroma traction in inspiration, for without it there would tend to be a narrowing of these tubes with elongation, and the deleterious effects of this on the respiration and circulation can well be imagined.

It might at first blush be thought, too, that in inspiration, particularly if forced, when more space within the thorax is being occupied by air in the dilated spaces, these spaces would expand at the expense of the blood vessels which would thereupon become crowded and narrowed, with a consequent impediment to the flow of blood within them.¹ Fortunately the normal state is quite the reverse, and

¹Professor Frederick R. Miller, F.R.S., tells me that the right heart becomes dilated if, in inducing artificial respiration with the thorax closed, air is forced into

the blood vessels are actually opened up with the expansion of their surrounding air spaces. The physical conditions which expand the air cells also expand their associated blood vessels synchronously. The stroma of extendable and retractable tissue is so arranged that the air and blood channels are pulled upon in the same manner and at the same time. In areas of actively respiring lung they all lengthen and widen in inspiration, and shorten and narrow in expiration.

Only passing notice can here be taken of the pulmonary capillaries in relation to respiratory movements. It has been shown that the capillaries are too large, so to speak, for the alveolar walls and bases in which they are situated, and are contorted, protruding into the alveolar cavity (Loosli, 1938). When the net is widened some of the slack is taken up, but the vessels are not narrowed. Thus in inspiration the caliber is undisturbed, or even enlarged. The action is not, however, as in the other blood conduits, due to stroma traction, but may rather be referred to a diminution of the pressure of air around their extremely thin walls. The capillaries might then become slightly swollen because of the relative increase in pressure of blood within in relation to the immediate environment. Further reference will be made to the capillaries in the discussion.

The rabbit's lung has alveoli of intermediate size, as determined by the careful measurements of Hartroft (Hartroft and Macklin, 1943b, 1943c). Those in this paper already described he has found to be larger, and those which follow, smaller. From the point of view, however, of influence on blood vessel caliber and length it would seem that the fundamental principles apply without regard to alveolar size. In all of these lungs the relation of the terminal venules, and indeed of all the branches of the pulmonary arterial and venous trees within the lung, to their environing alveoli, is on the same general plan, irrespective of alveolar size, and the same histological inferences as to functional collaboration may be drawn.

PLATE III

Monkey lung.

Fixation of this macaque lung was by vascular perfusion *via* the inferior vena cava in the unopened thorax. This technique reveals the arterioles well opened out, with walls much thinner than in the usual the bronchial system under too high a pressure. Under this condition it would be reasonable to expect that the air space would encroach upon the blood space. In normal breathing, however, both air and blood channels are free to expand in inspiration, and do so.

preparation by immersion of the block. Such an arteriole *Art* is shown in cross-section in A, lying between two well-distended alveolar sacs *as*; and it bears the same relation to them as do the venules. Bases of a number of alveoli with open outlines *a* are seen to make up the outer part of the wall, as in the venules. It is clear that the arteriole would be lengthened and widened by expansile movements of these air sacs just as would a venule under like conditions. Other alveolar bases in this figure abut upon neighbours like themselves, belonging to contiguous distended alveolar sacs, and are known as "partitional" bases (Macklin, 1944, 1945) in contradistinction to those of the marginal type already referred to. Lines of such partitional alveolar bases have a zigzag appearance *z*, when cut crosswise (Hartroft and Macklin, 1944a), and when the sacs are distended the kinks tend to straighten out as the bases gain area. The free ends, often knobbed, so frequently seen in this and other sections of these lungs, represent the partitions separating alveoli cut through the mouth so as to show the open outline (Hartroft and Macklin, 1944a). To the right in this photomicrograph is another arteriole with a fine dilated terminal twig emerging from it, and here again we have a clear view of the intimate relation of the contiguous alveoli to the vessel wall.

In B is seen a vein *v₂* with a sheath rather thicker than usual, made up of delicate fibers and tissue fluid spaces. Leakage of alveolar air into vascular sheaths such as this has been held to lead to an important clinical condition known as airblock (Macklin and Macklin, 1943, 1944). The endothelial surface is somewhat wrinkled, doubtless as a result of the dehydration technique. Entering this vein are two venules *v*, each surrounded by alveoli with intimately applied bases. These bases can be followed as a layer into those around the vein *v₂*, which form the outer boundary of its sheath. If such a membrane made of applied alveolar bases could be cleared of all adnexed tissue and spread out flat it would be seen to make a mosaic.

Guinea-pig lung.

In A a venule *v* is seen cut directly across. To the left is a dilated arteriole *Art* cut on a long slant, and partly filled with blood. The walls of both present a characteristic armature of distensible tissue *as*. In B we see bloodfilled branched venules *v* cut lengthwise, and a cross-section of the vein *v₂* draining them. Good views are afforded of capillary plexuses tributary to the venules. All of these channels are enveloped by air sacs *as*, the bases of whose alveoli are affixed to their walls and constitute for them an elongational and expansional inspiratory mechanism. The material was fixed by perfusion *via* the aorta.

Goat lung.

Branched venules *v* entering a vein *v*₂, all washed clear of blood, are seen enveloped by air spaces *as*. Nowhere could we find a more convincing demonstration of contiguity of vessel walls and alveolar bases. All vessels are held well open. Artifactual wrinkling is seen in the walls. As elsewhere, the alveoli are cut to show either open *a* or closed *a*₁ outlines. Fixation was by aortic perfusion.

PLATE IV

Dog lung.

In A we have a terminal blood vessel which, from its relations and morphology, is taken to be an arteriole *Art*, with walls thinned from the aortic perfusion technique. It is running parallel with an alveolar duct *ad*, above, and it shows a forking into its precapillary arteriolar branches. There are a few cigar-shaped muscle nuclei in the wall, at one point in a cluster. It is surrounded by inflatable tissue, and is subject to the same sort of elongation and widening in inspiration on that account as are the venules.

In B we see a cross-section of a venule *v*, situated amongst imperfectly inflated lung tissue. It is filled with blood, and had it not been for this blood content it would doubtless be smaller than it is, as the tension of the stroma would not hold it open. In life, however, the pulmonary blood vessels do not close up in the collapsed lung, for we know that the pulmonary circulation is maintained in pneumothorax. The vessels are, however, shorter and probably narrower in this condition, and the volume of blood contained in the pulmonary vascular trees would be less than in the distended lung. Fixation was by aortic perfusion.

The lung belonging to C was first collapsed and then filled with fixative by injection into the trachea. We see a cross-section of a well-opened venule *v* surrounded by distended air spaces. The bases of the adnexant alveoli have in some small places pulled away from the vessel wall proper, and this is doubtless to be attributed to stroma pull set up by the distending force of the entering fixative associated with contraction of the venule wall proper unsupported by a blood mass in the lumen. In other areas of this lung section, not shown in the figure, there is a thickening of the sheaths, particularly of the arteries, due to the circle of alveolar bases having been pulled away on account of distention of their surrounding alveoli. The same out-pulling of the ring of perivascular alveoli has been found to be quite striking in other material where the lung was filled with fixative, *via* the bronchi, to its greatest degree. This is taken to confirm the view

of a radial (vasifugal) pull of the stroma, particularly of overdistended lung tissue.

In D a small artery is shown cut across, below, and from it an arteriole branch *art* proceeds upward in longitudinal section. This is in the same intrabronchially fixed lung as C. Around the artery there is a sheath of soft connective tissue which has become thickened because the ring of bases was pulled away by stroma traction as the surrounding air sacs underwent distention. In the case of the terminal arteriole, however, the bases and arteriolar wall are blended into a very thin membrane. There is a little artifactual wrinkling here. The relation to neighbouring alveoli a_1 is well shown. This is a striking demonstration of the fact that these small pulmonary blood vessels are *not* collapsed, or even narrowed, when the surrounding alveoli are distended, but are, on the contrary, dilated.

A vein, v_2 , distended until the walls are thin, is seen in E, surrounded by filled air sacs. This lung was fixed by perfusion from the inferior vena cava with the thorax unopened. The hornlike projections above are venules, and they may each be followed above and to either side into their obliquely cut continuations *v*. Each of these shows beautifully the relation to alveolar sacs *as*, and alveoli, open *a* and closed a_1 . In such a complex as this we can visualize the distending lung tissue pulling upon the vascular walls to lengthen and widen them; and, contrariwise, we can imagine the stroma of the deflating lung as relaxing and allowing the vessels to return to their original lengths and widths. In this connection it has been noted that both pulmonary veins and arteries show many elastic fibers coursing longitudinally (Macklin and Macklin, 1942), and the recoil of these fibers would shorten the vessels in the expiratory phase.

The section labelled "Pup" shows the same distensible sleeve around a small vein v_2 . All parts are here, of course, underdeveloped. The walls of the air sacs *as* are relatively thick. Fixation was by aortic perfusion.

In this set of dog sections the same general idea is paramount, namely, that both arteriolar and venular terminals are wrapped about with distensible tissue so intimately that they must change in length and width under the domination of the swelling of the air-containing tissue around them.

PLATE V

Baboon lung.

This material is rather rare histologically. Traversing the figure we see a longitudinal section of a small vein *v* running into a somewhat larger one v_2 . It is proceeding away from the region of a bronchiole *br*

and pulmonary artery *art.* Distensible tissue, not so highly inflated as that of some other figures, lies on both sides. Fixation was by aortic perfusion.

Rat lung.

In A we see an arteriole arising from a branch of the pulmonary artery *art.*, cut lengthwise to an unusual extent. It serves as a useful comparison with the venule *v* and vein *v*₂ in B, from the same animal. In both the investment of distensible air spaces is striking. Fixation was by aortic perfusion.

Mouse lung.

The mouse had the smallest alveoli in Hartroft's series. Mouse A shows a branched venule *v* surrounded by these tiny alveoli. The wall of the venule is extremely delicate. It empties into the small vein *v*₂. B shows a cross-section of a very small venule *v* surrounded by inflated air cells. Above and to the right is a bronchiole. Both animals were fixed by perfusion *via* the inferior vena cava with the thorax unopened.

Pig lung.

A venule is seen surrounded by air sacs. The material was fixed by immersion after exsanguination so that all vessels are contracted and empty. This venule shows no internal elastic lamina, as do arterioles of the same size in this lung. This specimen is not completely comparable with other members of this series inasmuch as the surrounding alveoli are not well distended; but it does serve to show that the investment of inflatable tissue in the pig is like that of the other mammals studied and reacts on the blood vessels in the same manner.²

DISCUSSION

This study is fundamentally an histological one, histology being developed in the modern manner to embrace the functional implications of the parts considered, and also to range to the borderland of pathology. In the use of this method it is freely admitted that there were "modernists," such as Aristotle, even in the so-called ancient world. It is a necessarily limited study, being concerned primarily with the significance, to the terminal pulmonary venules, of their immediate environment of moving air sacs, in the normal, living, mammalian

²I wish to thank Major Walter Stanley Hartroft, R.C.A.M.C., formerly my research assistant under War Project CW532 of the Department of National Defence, Army, for this material, and also Miss Edna F. Cunningham, Miss D. Jean Smith and Mr. Charles Jarvis for aid in the preparation thereof. Mr. Jarvis made the excellent photomicrographs.

lung, but it goes a little farther than the venules and briefly considers the corresponding arterioles and other branches of the two pulmonary vascular trees from the same point of view; and allows itself to find, in the dynamics of this beautiful circumvascular mechanism, a key to the better understanding of the lesser circulation, particularly as related to the "aspiratory," "sucking" or "pumping" action of the thorax and lungs, commonly discussed in treatises on physiology (as Howell, 1940) and thoracic surgery (as Sauerbruch and O'Shaughnessy, 1937). For a presentation of the broader aspects of the lesser circulation the reader may find such papers as that of Tigerstedt (1903) helpful.

An imaginary lungfree lesser circulatory system

In an effort to gain a clearer understanding of the functional significance of this sleeve of expanding and contracting alveolar sacs let us imagine that all lung tissue has been dissolved away from the pulmonary blood vessels and that they exist alone in the pleural cavity, hanging nakedly in space in normal position and interrelations, with their blood flowing, but still acted upon by pressure variations caused by inspiratory and expiratory movements. First, what would be the effect of inspiration, or rather, the attempt at inspiratory movement? This would reduce the environmental pressure of the blood vessels and so would induce swelling, but because of the varying thickness of the walls the swelling would be unequal and largely confined to the capillaries, which would become much distended. This is very different from the even and orderly widening of these blood vessels in the normal lung in this phase. It seems unlikely that there would be material length change in the parts of the arterial and venous trees. Now elongation of the pulmonary arteries and veins is something of the highest importance; indeed it is something absolutely inseparable from inflation of normal lung tissue. We have found that the alveolar sacs are so intimately related to these vessels that they cannot expand without lengthening them. The picture presented by our hypothetical lungless pulmonary vascular system during the period of reduced pressure in the "inspiratory" phase would, then, be vastly different from that of the normal system in its meshwork of expanding air sacs; for, as we have seen, there is in this an orderly lengthening, by traction of the stroma, of the various branches of the two vascular trees along their original axes, and this is in keeping with the same sort of lengthening in the various related branches of the bronchial system.

Let us look, now, at "expiration" in the hypothetical system. The swollen capillaries would, if they had been able to stand the strain, return to their original caliber, but there would be little change in the

larger vessels which would probably contract a little with slight if any shortening. This picture, too, stands in strong contrast to that of expiration in the normal pulmonary vascular system, where, as we have seen, shortening with narrowing is the rule.

We have only to make for ourselves mental motion pictures of the lesser circulation in each of these contrasting conditions, and compare them one with the other, to realize in a very dramatic way the importance of the rôle of the circumvascular encasement of air cells on the form and function of the pulmonary vasculature. In beholding these we would, like Galileo in another connection, exclaim regarding the pneumatic motor sleeve "It does move!" And move it does, fortunately for the blood vessels, for through it they are automatically and rhythmically adapted to their duties of maintaining a proper blood flow under constantly altering conditions of volume and pressure, while keeping perfect step with their colleagues, the various branches of the bronchial tree.

Visualization of pulmonary blood flow

It has long been known that the systemic arterial blood pressure curve shows respiratory undulations whose hollows and peaks do not exactly correspond with the onsets of inspiration and expiration respectively, but occur a little after these points; and physiologists have gone to some pains to explain the curve in the light of what was thought to be happening in the heart and in the blood vessels, particularly those of the lung. Howell (1940) states:

It is generally agreed that the effect of the respiratory movements on the arterial pressure is due mainly to mechanical factors which influence the amount of blood discharged into the aorta. The matter is difficult to analyze successfully, but the following factors are the ones which have usually been emphasized. At each inspiration the aspiratory action of the thorax upon the venous flow to the right side of the heart is increased, and consequently more blood is thrown into the pulmonary circulation and eventually into the left ventricle. This factor would tend to increase the output of the heart during inspiration and thereby raise arterial pressure. On the other hand the blood-capacity of the lungs is increased during inspiration, owing to a stretching of the blood-capillaries during the expansion of the lungs, and this increase in capacity may serve, temporarily at least, to hold back the flow of blood to the left ventricle and thereby cause a fall of pressure during the inspiration. So far as these factors are concerned, it is evident that the permanent resultant effect should be in the direction of an increased flow of blood into the aorta and a rise of aortic pressure as a result of inspiration, but the time relations of this rise of pressure may be obscured or reversed by the temporary retarding effect of the increase in the capacity of the capillary bed in the lungs at the beginning of inspiration. There is an additional factor, however, whose influence is more evident as regards the time relation between the rise of aortic pressure and the phase of respiration. This factor is a change in heart-rate caused by the inspiration. In some individuals the heart-rate increases very perceptibly during inspiration, the change in rate taking place

quite promptly with the beginning of the inspiratory act. In others this phenomenon is less marked, or is absent altogether.

The foregoing explanation seems reasonable as far as it goes, but would probably apply more fittingly to the relatively passive conditions of the hypothetical lungfree pulmonary blood vessels than to those more active ones of the normal lung-engulfed system. Therefore the description should be altered to take into account the happenings in the arteries and veins, as well as the capillaries. These alterations can only be suggested now, and before we are in a position to make them final we will have to have the results of a great deal of further investigation by physiological and other methods. No experimental evidence has been here adduced apart from the different fixation techniques used. Because of the lengthening and widening of the arteries and veins in inspiration it is felt that the blood space within them is then enlarged materially. It is not known how much the capillaries dilate during inspiration; and recovery from any dilatation that did occur would probably be sluggish on account of their non-contractility. It may well be that the capillaries are protected from overdilatation by this perivascular ventilational mechanism which spreads the effect of reduced inspiratory pressure over the entire pulmonary vascular system rather than bearing down altogether on the capillaries, for if they had to stand all of this distensile force they might be injured.

This active enlargement of the arterial and venous volume, too, would take up part of the incoming blood at the beginning of inspiration, so reducing the amount then entering the left atrium, and occasioning a transient lowering of the systemic blood pressure. The quickened heart-beat at this time would more efficiently fill this space from the more readily available supply of blood from the abdominal veins. The conception of the streambed, widened through stroma traction, makes clearer the facilitation of pulmonary blood flow in inspiration, which is indicated in the rise of systemic blood pressure occurring during most of inspiration and continuing into the earlier part of expiration. The pulmonary arteries, as well as the veins, are more easily distensible and extensible than are the corresponding systemic vessels. They are equipped, too, with an adequate self-shortening mechanism of elastic fibers coursing parallel with the axis. The opening up of the pulmonary streambed in inspiration might be likened to diastole.

In expiration, we have a sort of systole, for the streambed of the arterial and venous trees is shortened and narrowed, with diminution in capacity, and this process is active, engendered by the elastic tissue, and, it may be, also by the smooth muscle of the vessel walls. The increased mass of blood so evacuated, on entering the left atrium at

the beginning of expiration, may be regarded as serving to augment the systemic blood pressure, and so to carry the peak into the early part of the expiration phase. The conception of prompt vascular recoil would seem to give a more credible explanation of the position of this systemic blood pressure peak in the expiration phase than the older conception of a possible recoil of the alveolar capillary walls.

It would be interesting to have accurate determinations of the volumes of the pulmonary vessels in inspiration and expiration under conditions of rest and exercise. We would then be in a better position to assess the value of the aid given to the right heart, if any, by such a filling and emptying process—a “pulmonic” propulsive cycle, with diastolic and systolic phases, if so it may be termed. The volume difference between the two phases might be very little in quiet breathing under sedentary conditions, but would undoubtedly be great in the young, healthy athlete in a state of prolonged violent physical exertion where the respiration is rapid and deep, and where filling and evacuation of the pulmonary vasculature is at a maximum. In such a case this mechanism might constitute a sort of accessory heart in that it would facilitate the flow of blood in the pulmonary circulation, and, consequently, in the heart and general circulation. Its value in providing for a respiratory and circulatory reserve is regarded as significant. Starling's *Physiology* (1941) states (p. 696): “At the height of inspiration the blood contained in the lungs is about one-twelfth of the whole blood in the body,” and that “this amount is said to be diminished during expiration to between one-fifteenth and one-eighteenth”; but it is the capillaries, apparently, that are being considered here.

Strenuous physical exercise

In strenuous physical exercise, especially if prolonged, there is an enormous demand by the tissues, particularly the muscles, for oxygenated blood, and to meet this there is a vastly increased action of the lungs. Greater gulps of air are taken oftener into the greedy pulmonic maw. This augmentation is not alone for the purpose of getting more oxygen into the blood and of eliminating more carbon dioxide. We must always remember that the lung—or more properly the pulmonary vasculature—is a bridge for the blood which must be crossed by sufficient amounts if the systemic vessels are to be properly supplied. It may seem trite to say that this bridge is strategic, vital indeed, but it may be worthy of a fresh inspection in the light of our histological findings. So much depends on the ability of this bridge to carry varying loads of blood—to increase its capacity when the need for that arises. It can broaden out, so to speak, when increased throngs of

corpuscles demand conveyance across; and not only that, this remarkable bridge can actually help those throngs to the other side, can hurry them along to make room for others, can rush them, like troops, to the front line, where they are desperately needed. This ability on the part of the lesser circulation to adapt itself to the greatly heightened demands of violent, prolonged physical exercise is bound up with its investment of continuously expanding and contracting air sacs, and with its innate distensibility and contractility; and this means that the adaptation is spontaneous, automatic. The lung itself controls it; the lung itself enlarges the streambed, widens the bridge, even imparts to it a sort of pulse—a lungbeat. This is but part of the wonderfully sensitive adaptational mechanism of the “Brustorgan,” built and operated to the end that the body may best adjust itself to the stresses and strains of strong muscular exertion. The volume of the pulmonary arterial and venous trees is automatically enlarged and diminished in the inspiratory and expiratory phases respectively, and in this way the efficiency of the bridge for the transmission of the increased load is raised. There must be no “bottle-neck” of traffic in the lesser circulation. Blood—sufficiently oxygenated—must be supplied in ample quantities to the systemic arteries. It would seem that this opening and closing action of the pulmonary vasculature, motivated by the lungs, under the governance of the medullary centre, goes far to ensure an adequate blood supply to the general circulation.

Applied physiology and clinical applications

If, however, the demands of the normal over-exerted body are too great there may be a breakdown of the bridge—or, shall we better say, a clogging of traffic. The blood is not moved along over the bridge fast enough; it accumulates in the great systemic veins, and the right heart dilates. The individual—human or race-horse or what-not—collapses. Recovery, through continuance of the vigorous pumping action of the thorax and consequent motivation of the pulmonary “accessory heart,” usually takes place. In this recovery, as much freedom as possible for the action of the breathing mechanism should be conferred. Basketball players, and other athletes, to “get their breath” after strenuous play, are said to lie on the back with the knees bent, breathing hard; and it may well be that this position is best for the free action of the chest walls and diaphragm, while keeping the systemic circulation at ground level. Oarsmen, after a gruelling race, are said to heave their chests violently while slumped over the oars, but this posture may be the only one available and not ideal. The great thing is to keep the bridge as open as possible to guarantee an ade-

quate passing over of blood from the right to the left side of the heart. With freedom to act, individuals instinctively do the best thing possible, but if unconscious they must be aided in finding the best posture. In that extremity, artificial respiration should help in resolving the traffic snarl of right heart and venous congestion.

Over-exertion

What occurs if this bridge becomes overly clogged? It has been surmised that Phcidippides, for instance, after his twenty-six mile first Marathon, died suddenly because his bridge was inadequate under the circumstances to meet the prodigious demands upon it and hence the blood was dammed back on his right heart, over-dilating it; and that this unfortunate emergency was precipitated by his making a speech, announcing the great victory, short though it reportedly was. Pausing to speak, instead of vigorously working his chest in the most advantageous posture, he interrupted his pulmonary bridge traffic, with disastrous results. We shall never be able, of course, to settle this point, but the example serves to illustrate the all-importance of maintaining the pulmonary blood thoroughfare under all circumstances. The normal "thoracic organ" has amazing reserve potency, and can take terrible punishment, as is shown strikingly, for instance, in the endurance of troops, like the commandos, when undergoing modern training. When men go "at the double" for miles with heavy equipment until they fall exhausted, and then get up as soon as possible to plunge along again for miles, and do this again and again, even under a boiling sun, it is evident that their pulmonary bridges are carrying the increased traffic successfully. This part of lung physiology is worthy of even greater attention than has so far been given it. The kinetics of the pulmonary blood vessels should be surveyed from all possible angles, not omitting a study of the capillaries. Roughton (1945) has found that the average time, t_L , spent by a red corpuscle in passing through the lung capillaries is reduced in hard work as much as 7.5 times, and may be as little as 0.1 second. He also finds, calculating from t_L , that the total volume of the blood in the patent lung capillaries in normal men averages 60 cc. at rest and 95 cc. in hard work, and this indicates to him that no very extensive opening up of extra capillaries occurs in the lungs during exercise. Although this volume was not calculated for the specific periods of inspiration and expiration, the results do not lead us to think of any great distensibility on the part of the lung capillaries.

Abnormal circulatory conditions

Only those pulmonic circulatory events which transpire in the healthy human subject or animal have so far been considered, and it

is possible only to touch upon those of the abnormal lung. There is a wide field for study here. Reactibility to the influence of the adnexed air sacs may be lowered in the two vascular trees and in the associated bronchial tree, and this deficiency will mean a decline in the respiratory volume fluctuation of the blood vessels as of the bronchi. Broncho-sclerosis (Macklin and Macklin, 1942) is an actuality and involves the neighbouring blood vessels in movement restriction. Pulmonic arterio-sclerosis similarly affects the freedom of movement of the vessels as well as that of associated lung tissue. It may be that the beneficial effect of inspiratory widening of the pulmonary blood vessels is primarily to offset the disadvantage of the narrowing which, without the centrifugal pull of the stroma, would occur, but its rôle in opening up the streambed in proportion to the need for increased blood flow seems firmly established. A similar condition obtains in the air channels whose capacity, in strenuous exercise, is increased. Without the traction of the stroma they would collapse. How otherwise are the non-cartilaginous bronchioles and alveolar ducts held patent?

In chronic alveolar ectasia (the so-called "medical" emphysema) the blood vessels are more or less set in an elongated state, along with the bronchi, and this means a curtailment of range of respiratory movement. In this disease the air sacs are dilated, deformed and inelastic, and the makeshift for ventilation in them may involve little or no stretching of their walls, but only an approximation and separation of these, much as in the filling and emptying of a paper bag. But even this weak substitute for the usual elastic process may have some effect on the morphology, as well as the function, of the attached arterial and venous branches. The blood vessels may not be sufficiently widened, because of a decrease in the amount of pull of the lung stroma. Deterioration of the accessory heart action of the pulmonic vasculature may well account for some of the shortness of breath so characteristic of this malady after even moderate physical exercise. Taking the lung as a whole, even if the pleura were inextensible but still sparingly flexible it might be possible to secure some slight ventilation by a simple flattening and widening process in the air sacs as above mentioned; but the lung is not a simple bag, it having a complicated internal structure or framework which normally adapts itself to inflational and deflational processes (Macklin, 1932a, 1932b), so that ventilation, without a certain amount of morphological alteration of this interior framework, of which the two vascular trees make up a large and important part, would not be possible. Emphysema should be re-examined from the angle of impairment of vascular function. Such deteriorations as those mentioned would mean a serious loss in circulatory and respiratory reserve power.

Indeed the pulmonic vascular bridge should be examined in all relevant pathological conditions to see what inadequacies, if any, it presents. How is this bridge affected, for instance, in lobar pneumonia? The consolidated region is stiff, and length and width changes in the vessels thereof would be impossible. The accessory pulmonary heart would be impaired in consequence. What of the still functional part of the lung? Would its vessels perform their functions normally? Is the part of the streambed in the functional area of the lung distended, with, perhaps, a more marked pump action? How, if at all, is the bridge influenced by toxæmia? Are there ways and means of assisting the hemic traffic of the bridge?

Or, take the simpler case of atelectasis from occlusion of a bronchus by a foreign body. The vessels of the affected part, because of the collapse of their air sac investment, would be shortened and narrowed, with diminution of volume (Coryllos and Birnbaum, 1929). This part of the pulmonic vasculature would show lessened pumping action. In the surrounding compensatorily emphysematous region the vessels would be widened and lengthened, carrying relatively more blood. The enlargement of the streambed here would also be compensatory. Thus we find compensatory emphysema engendering compensatory facilitation of the circulation in this part of the lung. The practical value of the compensatory emphysema is the widening of the streambed, rather than the air bed. Would the net capacity of the bridge be altered, with reduction in one part and increase in another? From such a problem case one might go on to the consideration of atelectasis in general, from the same point of view.

In tuberculosis³ the situation is more complicated, but it is very important that we understand the state of the bridge in this as in all other conditions. Atelectasis and compensatory emphysema occur here too, along with actual degeneration of parts of the bridge from disease. How would the bridge be affected in asthma, for instance? We know that quite a few of these cases have right-heart failure. In these, how is the bridge narrowed, if at all? Edema of the vascular sheaths is known to occur in some conditions, such as those arising from exposure to irritant fumes; and it seems evident that this would involve a narrowing of the affected vascular channels with a corresponding handicap to bridge traffic. The finding of associated right-heart blockade confirms this judgment. Even in simple ageing, involving as it does a certain amount of stiffening of the pulmonic sub-

³I wish to thank Professor David W. Crombie, M.R.C.S., L.R.C.P., Superintendent of Queen Alexandra Sanatorium, London, for many helpful suggestions, and for his inspiring interest and encouragement.

stance, we may expect a cutting down of respiratory and circulatory reserve on account of diminution in bridge capacity. In all cases with right-heart dilatation the bridge should be studied to see if there are ways and means of opening it up to more traffic. Sudden complete or partial obstruction of the bridge occurs in pulmonary embolism (Hall and Ettinger, 1933). An embolus in the pulmonary artery would probably lodge farther on because of the inspiratory widening of the channel.

The systemic accessory heart

In stressing the action of the allied air sacs and blood vessels in helping the blood in its course over the pulmonary bridge it is not intended to overlook the systemic hemic bridge which must be crossed by the blood in travelling from the left to the right side of the heart. Here the great impulsive mechanism is the active musculature, and there is no doubt that, in the vigorously exercising body, the blood is aided in its passage through the veins by environmental pressures and the favourable placement and design of the valves. This side of the hemodynamic picture should be looked at carefully in such interesting subjects as the long-distance runner and the speeding race-horse, but the temptation to do so at the moment must, regretfully, be resisted. We may allow ourselves to reflect, however, that there is a place where these two bridges come end-to-end, and that is the mediastinum.

The two blood bridges

Though we know that each of these bridges is, in itself, circular in the sense that a circuit is traced by the blood in going over it, we may, diagrammatically, consider each bridge as a semicircle, the two forming a circle with systemic-pulmonary and pulmonary-systemic junctional regions. For analytic purposes we may find it instructive to look at the blood-stream in the former junctional region, for it is here that a traffic-jam occurs when the pulmonary bridge becomes inadequate; and to recognize here two parts of the course, (1) that leading from the systemic veins into the heart, and (2) that leading from the heart into the lungs.

Additional histological evidence

Besides the material already referred to, hundreds of serial sections from specially prepared lungs of six rats and two cats added confirmation to the generalizations that have been made regarding the envelope of expansile tissue around the pulmonary blood vessels, and the same can be said for a large collection of lung sections of human and lower mammalian lungs prepared for other studies by many different me-

thods. Casts of the vascular system of comparable lungs in contrasting states of expansion are being prepared, and other techniques are being brought into operation in an effort to provide still more evidence bearing upon the problem herein discussed.

Fixation technique

Although different methods of fixation were used, the pulmonary arteries and veins were always found in the sections to be patent as long as the surrounding air cells were distended. Over-filling of the free lungs with fixing fluid does not collapse these vessels but, on the contrary, dilates them, as does over-filling with air under the same conditions. Sections from lungs fixed by perfusion of the pulmonary blood vessels with preserving fluid did not show indications of pressure by the vessel wall upon the neighbouring air sacs, and it seems reasonable to conclude that this method did not unduly dilate these vessels. In lung material from the pig, after exsanguination and fixation by simple immersion, areas still retaining air show patent blood vessels, though the degree of openness is less here than it is after fixation by vascular perfusion or bronchial filling.

SUMMARY

The pulmonary venules of normal lungs in eleven species of mammal, including man, were found to be enveloped by air cells in such a way that expansion of these cells would lengthen and widen the vessels and contraction of them would allow the vessels to become shortened and narrowed through the recoil of the elastic fibers of their walls. The same is true of the pulmonary arterioles and the larger branches of the pulmonary arterial and venous trees.

This morphological complex, in respiratory action, in the young healthy mammal, is characterized by an increase in the volume of the pulmonary arteries and veins in inspiration and a decrease in expiration. The range of volume variation in these vessels is greatest in forced breathing, as in prolonged vigorous exercise. Such a movement of the functionally interacting air and blood tracts of the lung is pump-like, and is regarded as of value in moving the blood from the right to the left side of the heart. The mechanism may be thought of as a pulmonary accessory heart with inspiratory diastoles and expiratory systoles.

Viewed in this light the pulmonary blood vessels are conceived of as a bridge whose carrying capacity is varied by means of this respiratory action of the circumvascular air cells to meet the physiological need of the moment, and whose channel, by the movements of the

enviroming lung tissue, particularly in vigorous exercise, has an actual propulsive effect on the blood which is being carried across it.

A traffic-jam of serious nature can occur in the region of entrance to this bridge—the right side of the heart and associated systemic veins—in the normal but physically overworked subject.

In recovering from too great physical exertion the individual should be placed in the position most suitable to the favourable operation of the bridge, and that would seem to be on the back, with knees drawn up. The breathing should be deep and rapid. Artificial respiration should be used when necessary for the better operation of this pulmonary vascular bridge.

Decline in efficiency of this bridge, or pulmonary accessory heart, is discussed for a number of disease conditions. Shortness of breath in emphysema, for instance, is attributed to such a decline. Intensive study of this bridge in all relevant conditions is enjoined. The reserve value of the normal bridge is stressed.

Because the expansile force of the incoming air is spread, by means of the perivascular sleeve of air cells, over the pulmonary arterial and venous systems, rather than being concentrated upon the pulmonary capillaries, as in the older conception of the action of "negative pressure" on the intrathoracic circulation, it is felt that the capillaries are protected from possible harm. Were the capillaries alone exposed to the pressure difference of inspiration they would probably be unduly distended and even ruptured. The opening out of the entire pulmonic arterio-venous channel by vasifugal stroma pull effectively lowers the peripheral resistance.

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EXPLANATION OF PLATES

PLATE I

Human.	S80-2H-5LU-10.	10u.	Hematoxylin and Eosin.	X50
Cat A.	S76A-17C-1RU-9.	10u.	Hematoxylin and Eosin.	X50
Cat B.	S76-4C-4RC-13.	10u.	Mallory-Azan.	X50
Cat C.	S76-4C-4RC-5.	25u.	Hematoxylin and Eosin.	X50
Cat D.	S76-4C-4RC-7.	10u.	Masson's Trichrome C.	X50

PLATE II

Rabbit A.	S76-6R-2RM-9.	10u.	Hematoxylin and Eosin.	X50
Rabbit B.	S76-3R-3RL-9.	10u.	Hematoxylin and Eosin.	X50
Rabbit C.	S76-3R-3RL-10.	10u.	Hematoxylin and Eosin.	X50
Rabbit D.	S76-6R-1RU-8.	10u.	Masson's Trichrome C.	X50
Rabbit E.	S76-3R-8RU-3.	25u.	Hematoxylin and Eosin.	X50

PLATE III

Monkey A.	S73-22M-4RC-14.	10u.	Mallory-Azan.	X50
Monkey B.	S73-22M-4RC-10.	10u.	Hematoxylin and Eosin.	X50
Guinea-pig A.	S76-12G-2RM-8.	10u.	Masson's Trichrome C.	X50
Guinea-pig B.	S76-14G-3RL-14.	10u.	Mallory-Azan.	X50
Goat.	S76-5Gt-7LL-9.	10u.	Hematoxylin and Eosin.	X50

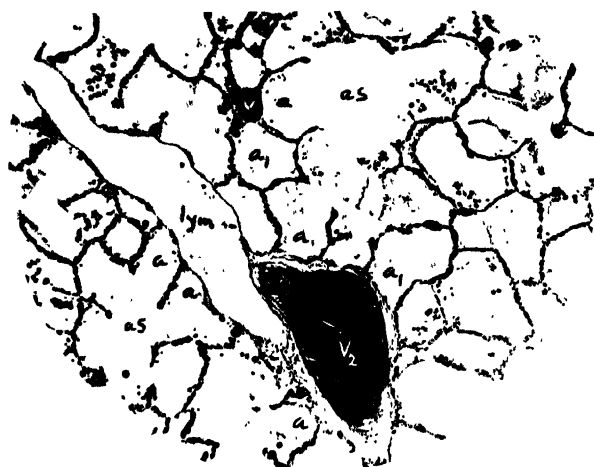
PLATE IV

Dog A.	S76-13D-5LU-9.	10u.	Hematoxylin and Eosin.	X50
Dog B.	S76-15D-3RLB-10.	10u.	Hematoxylin and Eosin.	X50
Dog C.	S74-3D-1RL-8.	10u.	Masson's Trichrome C.	X50
Dog D.	S74-3D-1RL-10.	10u.	Hematoxylin and Eosin.	X50
Dog E.	S73-21D-1RU-9.	10u.	Hematoxylin and Eosin.	X50
Pup.	S77-3P-5LU-9.	10u.	Hematoxylin and Eosin.	X50

PLATE V

Baboon.	S76-11B-3RL-8.	10u.	Masson's Trichrome C.	X50
Rat A.	S76-8Rt-3RL-14.	10u.	Mallory-Azan.	X50
Rat B.	S76-8Rt-4RC-14.	10u.	Mallory-Azan.	X50
Pig.	10b.	25u.	Weigert elastic.	X50
Mouse A.	S73-17Ms-3RL-9.	10u.	Hematoxylin and Eosin.	X50
Mouse B.	S73-16Ms-1RM-9.	10u.	Hematoxylin and Eosin.	X50

PLATE I



HUMAN



CAT A



CAT B



CAT C



CAT D

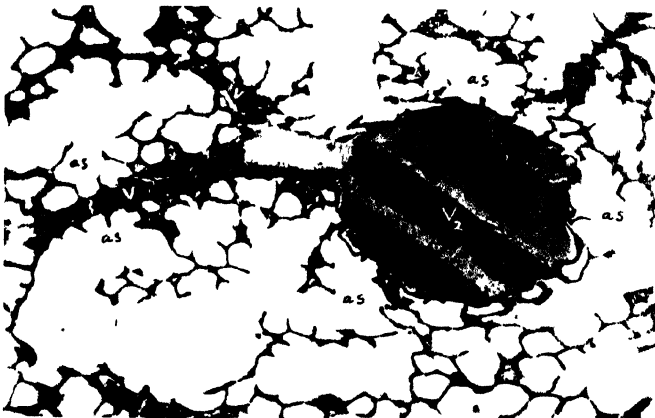
PLATE II



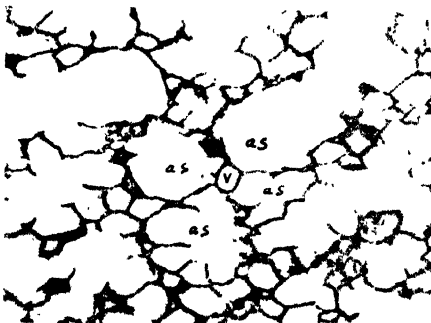
RABBIT A



RABBIT B



RABBIT C



RABBIT D



RABBIT E

PLATE III



MONKEY A



MONKEY B

GUINEA-

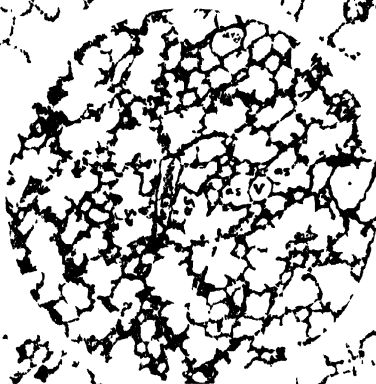
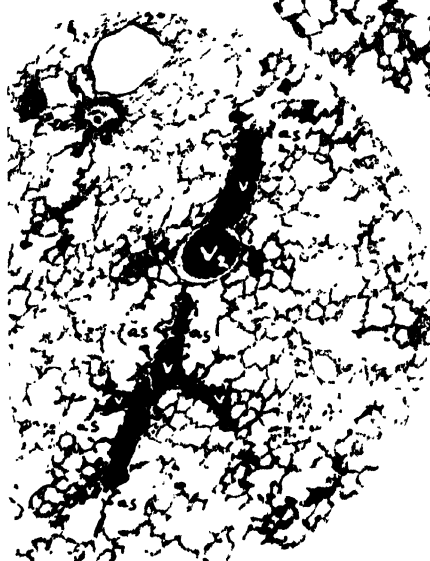
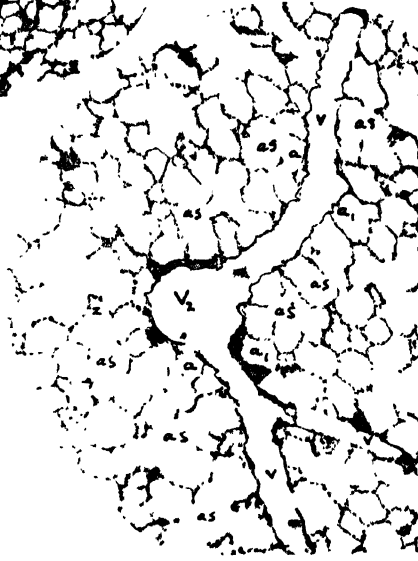


FIG A

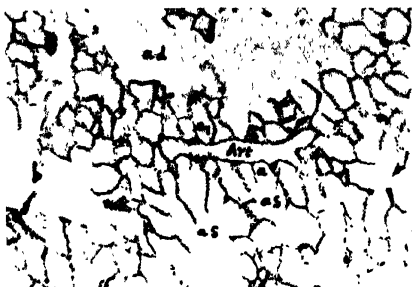


GUINEA-PIG B

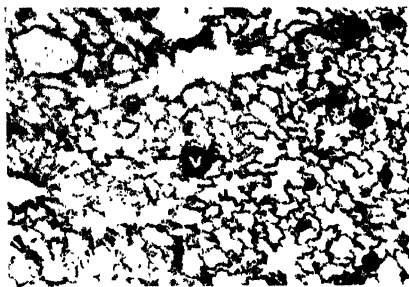


GOAT

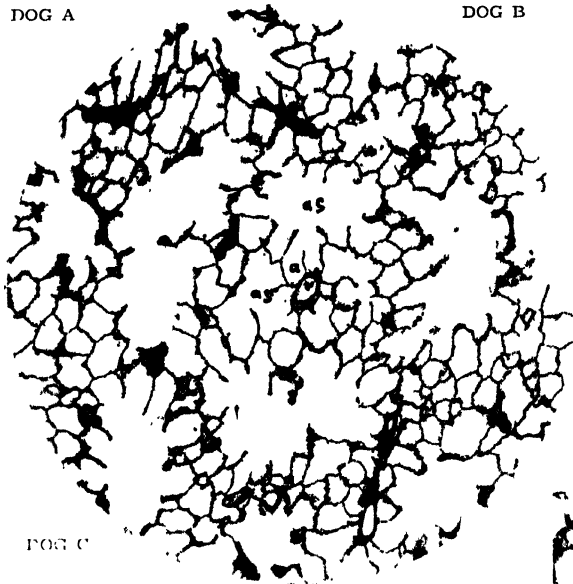
PLATE IV



DOG A



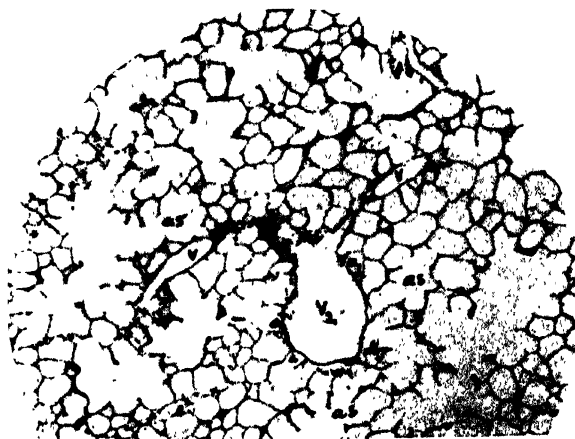
DOG B



DOG C



DOG D



DOG E



PUP

PLATE V



REPORT ON THE SPORE AND POLLEN CONSTITUENTS OF A PEAT BED IN THE SHIPSHAW AREA, QUEBEC

By NORMAN W. RADFORTH

Presented by R. B. THOMSON, F.R.S.C.

IN 1926, Vaino Auer, the pioneer investigator of peats in south-eastern Canada, made an extensive study of thirty-four peat bogs located between the Atlantic coast and the base of the Niagara Peninsula. In the most recent record of his work (Auer, 1930) it is stressed that "the aim was to investigate as large a region as possible in order to determine what were the general conditions pertaining to the peat bogs of this region and to outline the problems awaiting detailed investigations." A year after this work was published, Bowman (1931) made a detailed study of a single peat bed near the Matamek River, Quebec. The writer has no knowledge of any other published results of peat bog investigations made in the region in which Auer worked, and it may be added that, except for recent investigation by Wilson (Wilson and Webster, 1943) west of Lake Superior, peat studies have been almost entirely neglected in Canada in the past dozen years.

In the United States, research on peat has been maintained and the results are significant from several points of view. In that country, knowledge of the history and past distribution of the modern flora has been greatly increased; aid in the solution of glacial and post glacial geological problems has been provided; evidence facilitating interpretation of Pleistocene climate has been presented, and lately, engineers have realized that matters pertaining to foundation engineering and soil mechanics in certain instances may be affected by peat investigations (Matthes, 1945).

The inspiring preliminary work of Auer along with the reality that there is an unfortunate general lack of knowledge concerning the origin and constituents of Canadian peats, aptly emphasizes the need for scientific exploitation of this field in Canada. Indeed, the usefulness of such work in the United States makes the need in this country an urgency.

The present work has been undertaken with the hope that the results will be of some value botanically and geologically, and will be of interest to those in the field of applied science. In connection with the latter it should be stated at the outset that the peat samples on which this preliminary undertaking has been based were collected by

an engineer, Professor Robert F. Legget, Department of Civil Engineering, University of Toronto, whose interest in the undertaking has encouraged the author from the beginning.

Location and Extent of Peat Bed

The peat bed was discovered during excavation at a dam site on the property of the Aluminum Company of Canada, Ltd., west of the mouth of the Shipshaw River which drains into the Saguenay near Arvida in the Lake St. John district, Quebec (Fig. 1).

As indicated in the diagram of a section through the peat and associated beds (Fig. 2) all the Pleistocene is contained in a Precambrian basin. The peat itself is buried by about ten feet of silt and rests on an undersilt of approximately the same depth. The latter overlies sand and gravel which is limited by the Precambrian floor. The peaty layer contrasts sharply with the silt, and is here about two feet in thickness, with very little variation. Its area is at present not known exactly, but the silt which overlies it at the present exposures forms an extended plain implying that the peat also might cover a correspondingly large area. Part of the plain is shown in the photograph (Plate I, Fig. 1) and the relationship of the peat to its associated layers is seen in Plate I, Figs. 1 and 2.

In Plate II, Fig. 1, a photograph of a sample removed from the peaty layer, macroscopic constituents are in evidence. Casual examination reveals both gymnosperm and angiosperm branches and many twig and leaf fragments. In the bed the occurrence of tree stumps *in situ* has been noted (Legget, 1945). Detailed examination of these macroscopic constituents is underway, and will be dealt with elsewhere.

For the present, attention has been devoted to a study of the microscopic (spores and pollen grains) constituents of the bog, for it was felt that such a study would provide the surest way of designating the peat, and at the same time offer some means of ascertaining the horizon to which the peat belongs. Also, spore and pollen analysis facilitates correlation with other peats on the continent.

MICROFOSSIL ANALYSIS

Description of Peat Sample and Method of Analysis

The analysis was made from a single large sample of peat marked "No. 5 D.S." This was obtained by channeling down a fresh peat face. In this operation care was taken to give approximately equal repre-

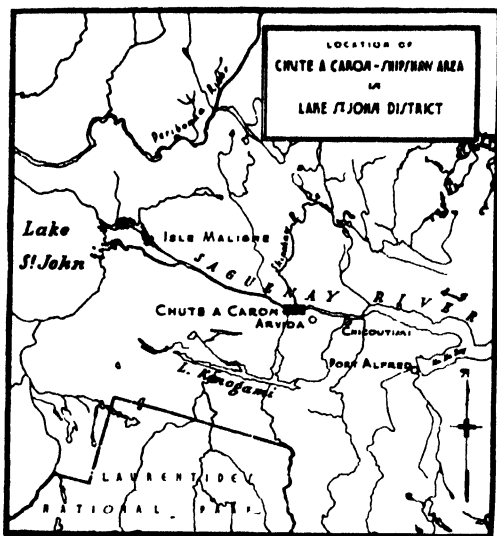


FIGURE 1.—Map showing the location (black rectangle) of the Shipshaw area.

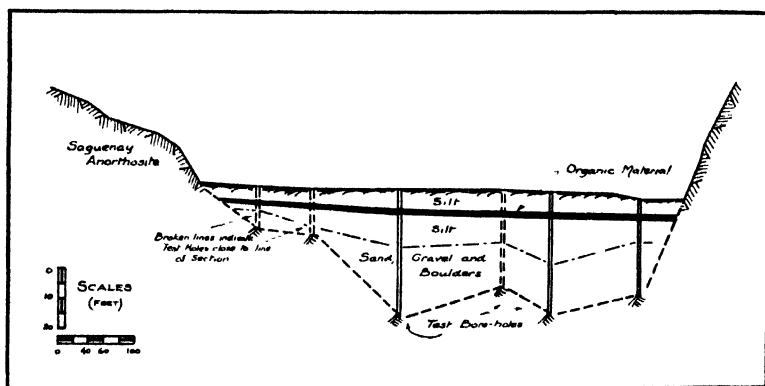


FIGURE 2.—Section through the peat bed showing the relationship of the latter to associated rocks at dam site No. 5. (Diagram also used by Legget (1945)).

sensation in the sample to each level in the seam. To ensure against contamination from living vegetation of the region, the sample was placed in a sealed jar.

The procedure usually adopted is to store the samples in such a way as to permit the analyst to investigate successive levels each a few inches in depth. When the present sample came to the attention of the writer it had already been mixed, making successional studies of the Shipshaw peat impossible unless further sampling appropriate to the more intensive study could be made.

The entire sample, apart from small twigs and other woody fragments easily isolated, consisted of a uniform, loose, dark brown concentration of organic matter aggregated in rounded lumps which varied somewhat in size. The largest lump was roughly one and a half inches in diameter.

The sample was turned out of the container and methodically mixed. Random selection of a dozen variously sized lumps was then made, and the surfaces of these were removed to the extent that each lump finally measured about 1 cm³. The material was then ready for maceration, deflocculation and mounting for study. The former two processes were investigated with other samples in preliminary experiments using various methods. Bromine was tried as a macerating and oxidizing agent. Alcohol, as suggested in Wilson (Geisler's schedule) (1944), and potassium hydroxide, used as recommended in an account by Sears (1930), were also tried. The latter gave excellent results in preparations made according to the description which follows.

The entire dozen cubes of peat were boiled in 40 cc. of 10 per cent potassium hydroxide for forty-five minutes. On cooling, the mixture was washed with water and centrifuged. The residue was again boiled in 10 per cent potassium hydroxide for thirty minutes, washed and centrifuged a second time. The residue was collected in four centrifuging tubes. Six mounts of the material were then prepared from each of the test tubes making a total of twenty-four mounts. As the mounting medium, corn syrup was preferred for the same reasons expressed in Radforth (1938). For examination four slides were chosen from the twenty-four mentioned above. Care was taken in making this selection to choose mounts in which distribution of organic matter was uniform, and in which the amount of material appeared to be the same in each slide. In making this selection, it was felt that the character of the original sample would be adequately expressed.

These four slides served as the basis of analysis for forest type pollen and for the pollen and spores of herbaceous plants most commonly

represented. The remaining twenty slides were used as special reference material in connection with the identification of individual pollen or spore types, and, with aid from the literature (Meinke, 1927; Sears, 1930; Wilson, 1934, 1944), for practice in microfossil identification in general.

A collection of pollen and spore mounts made from modern plants also served for reference and as a basis for comparison with the fossils, thus facilitating identification of the latter and rendering the results the more reliable.

For percentage and frequency determinations made from the four selected slides, a mechanical stage was used. In formulating rules for counting and identification, extreme care was taken to watch for possible sources of error which would have a serious bearing on the results. The counting method adopted was essentially similar to that used by Wilson (Wilson and Kosanke, 1940). For computing percentages of tree pollen fifty examples of these were identified from each of the four slides. For frequency determinations pollen and spores from herbaceous types were included in the counts.

Fossil Flora

The possibilities of error, and the difficulties encountered in avoiding them are great in microfossil statistical analyses in spite of careful application of methods. Several peat analysts, Erdtman (1943), Fuller (1935), Sears (1935), Wilson (1944) have indicated the nature of these difficulties and the consequent limitations to be placed on the results of analyses. These difficulties need not be enumerated again here; in spite of the limitations they set, the purpose of peat investigations is usually attained, a point receiving ample support from accounts of peat analysts everywhere.

The relative abundance of tree pollens, expressed as a percentage of the total two hundred examples, is shown in Table I. The frequencies with which these pollens occurred per cm² of mount are also expressed in Table I. Frequency of occurrence per cm² of mount for non-tree pollens and spores is given in Table II.

Listed under Filicales in Table II is the type described as spore "a." Examples of the latter were compared with spores of various living Pteridophytes. These comparisons point to a possibility that spore "a" is the spore of *Pteridium aquilinum* (L.) Kuhn, but a decision on this point must be delayed until further investigation is completed. Regardless of the ultimate designation of the spore, it is useful to the writer as an important index fossil because of its high frequency.

Some microfossils have been listed in Table II as unidentifiable. This group contains examples which were modified or broken either during fossilization or in maceration to a degree which rendered their identification too difficult.

TABLE I

	Genus	Relative Frequency %	Frequency per cm ² of mount
Tree Pollen	Gymnospermæ	<i>Abies</i>	30
		<i>Juniperus</i>	05
		<i>Larix</i>	85
		<i>Picea</i>	80
		<i>Pinus</i>	62.5
		<i>Thuja</i>	10
		<i>Tsuga</i>	3
	Angiospermæ	<i>Alnus</i>	45
		<i>Betula</i>	30
		<i>Carpinus</i>	2.5
		<i>Cornus</i>	0.5
		<i>Corylus</i>	35
		<i>Juglans</i>	0.5
		<i>Ostrya</i>	0.5

TABLE II

	Spore type	Frequency per cm ² of mount
Non-Tree Pollen and Spores	Lycopodiaceæ	
	<i>Lycopodium</i>	20
	Filicales	
	<i>Polypodiaceæ</i>	34
	Spore "a"	106
	Other Pteridophyta	15
	Crinoidspores	24
	Unidentifiable	39
Trees of Modern Flora	TABLE III	
	Genus	%
	<i>Picea</i>	50
	<i>Pinus</i> (strobilus)	5
	<i>Betula</i>	30
	<i>Alnus</i>	5
	<i>Populus</i>	5
	Others	5

Modern Flora

Examination of the photographs, Plate I, Figs. 1 and 2, suggests that the existing tree flora in the peat area is predominantly spruce. Through the kindness of Charles Miller, Resident Engineer, Aluminum Company of Canada, Ltd., a survey has been made of the tree flora growing within a radius of two miles of dam site No. 5 where the peat sample was found. The results are shown in Table III. While the relative percentages expressed in the table are only approximate there is no reason to doubt that, as suggested in the photographs, spruce is the predominant tree type existing today.

DISCUSSION AND CONCLUSIONS

Amounts of Tree Pollen

The results expressed in Table I indicate that the tree flora represented in the peat was largely coniferous. The relative frequency of 62.5 per cent intimates predominance for *Pinus*. It should be emphasized here, however, that this frequency and that determined for other constituents are representative of the entire depth of peat, not of any one level in it. Thus, because the sample from which the data have been derived contains all levels of the peat, the spectrum expressed in the relative frequencies gives the flora of a forest over the latter's entire history rather than at any one short phase in its history. It follows that, because relative frequencies for *Picea* and other genera are so low by contrast to that of *Pinus*, there is little likelihood that any of the low frequency forms predominated at any phase in the history of the forest.

A point requiring separate consideration, one arising from a study of the results in Table I, centres on the validity of the apparent occurrence of *Larix*, *Juniperus*, *Thuja* and *Populus* pollens. Some analysts claim that cuticles of these grains are rare in peat chiefly because conditions under which peats are formed are not conducive to their fossilization (Cain, 1939). Although the writer was unable to find any pollen of *Populus*, he was satisfied to designate certain grains as *Juniperus*, *Larix* and *Thuja* in the proportion shown as frequency per cm² of mount in Table I. These pollen grains can be located for future reference in case it is felt that their identification requires further confirmation. The importance of these pollens, apart from their rôle in characterizing the Shipshaw peat, is not great in any case, owing to the great preponderance of those of *Pinus*. It is on the basis of these and the other less problematical tree pollen types listed in Table I that the most significant conclusions will be drawn.

Amounts of Non-Tree Pollen and Spores

In peat analyses little importance is given to microfossils of the sort listed in Table II. Perhaps, as suggested by Meinke (1927), this is unfortunate. It is the hope of the writer that this list will be expanded in future work on the Shipshaw peat. In the meantime, the types and their frequency figures shown in this table, along with those given in Table I, provide a reasonably good index for the Shipshaw peat, and serve basically in facilitating correlation with other peats. Spore "a" (*Pteridium*?) by reason of its great abundance should be particularly useful in this regard.

It will be noted that in Table II, the frequency number for Angiospermae is 24. This figure is an aggregate of frequency numbers for the following families: Cyperaceae, Gramineae, Nymphaeaceae, Ericaceae, and Compositae.

The frequency number 34, Table II, for microfossils which are classed as unidentifiable might be significant for reasons already expressed in connection with frequency numbers of non-tree pollens and spores. In addition, this number might be useful in estimating degree of improvement of technique in future work; if it is lowered appreciably with a modified technique, the latter would probably be regarded as more desirable than the present method.

Comparison of Peat Flora with Modern Flora

A comparison of Table I with Table III reveals a contrast between the peat flora and the flora now characteristic of the Shipshaw region. In the ancient flora, *Pinus* predominated and *Picea* was in distinct minority, whereas in the modern forest the reverse is true, so that *Picea* prevails and *Pinus* is in a minority. Until further knowledge regarding the spectra at various levels of the peat is at hand, it is perhaps unwise to draw other comparisons. In any case, the significant contribution lies in the *Pinus-Picea* contrast noted above.

Allowing for local environmental factors which often affect broad ecological decisions, it is usually accepted that pine predominance is indicative of a climate which is less moist and warmer than that in which spruce would prevail. It seems reasonable to conclude therefore that the Shipshaw peat was formed in a less humid and slightly warmer climate than exists on the whole in the region today.

This view is difficult to confirm from microfossil studies except indirectly, and in a partly theoretical sense. Spruce dominance, frequent in early postglacial history, often precedes an increase in pine. Von Post's hypothesis (1930) dealing with postglacial climatic fluctuations generally receives support from microfossil analysts. In it, it is postulated that postglacial time commences with a period of increasing warmth which might account for the increase in pine mentioned above.

Bearing of Results on Geological Interpretations

A knowledge of the climate prevailing at the time the Shipshaw peat was formed is of some help in determining the geological age of the peat provided the information is accompanied by other evidence of a stratigraphical and physiographical nature. The latter comes from helpful information now being recorded by Legget (1945).

At the moment of writing it is not possible to determine with certainty from geological data whether the beds in which the peat was discovered are glacial or postglacial in origin. Evidence significant in the solving of this dilemma comes from a comparison of the Shipshaw peat flora with the flora of other peats of known horizon.

Investigators of postglacial peats of northern United States and Canada reveal that frequently *Picea* pollen is in great abundance. Smith (1940), who studied 148 pollen profiles, draws attention to the fact that "a spruce maximum occurs near the bottoms of most of the older profiles." Following later is a pine maximum, and then a rise in deciduous types. These, Smith refers to as early postglacial. Finally, in the north, there is a return to a spruce maximum.

An inspection of this sequence reveals a phase in forest history with which the phase represented by the Shipshaw fossil flora might be favourably compared. On the other hand, if, on this basis, the Shipshaw peat is accepted as postglacial, its pollen spectrum would appear to be somewhat unique. One other region, mid-Wisconsin, shows a postglacial pine-predominant flora (Wilson and Webster, 1942) not unlike the Shipshaw tree flora. But this is considerably south of the latter, and its modern flora, also pine-predominant, contrasts sharply with the present Shipshaw forest. At a more northerly latitude, Wilson and Webster (1943), in their splendid work on the analysis of four peat bogs in the south-western portion of Northern Ontario, have shown that in two of the bogs *Picea* is distinctly predominant. In the other two bogs, although *Pinus* is predominant at some levels, *Picea* pollen is very numerous with a low in one bog of about 25 per cent and in the other of about 18 per cent. These last two figures, each representing an extremely limited region of the bog, are more than twice as great as the relative frequency number for *Picea* pollen in the Shipshaw spectrum where the frequency is representative of the entire depth of the peat. Bowman (1931) has examined the fossil flora of a postglacial bog some three hundred miles almost due east of Shipshaw. At all of the twenty-three levels he studied in the 11½ feet of peat, *Picea* pollen is predominant over *Pinus* pollen. Auer (1930) made determinations of the constituents of three bogs a few miles north of Rivière-du-Loup on the south shore of the St. Lawrence River. In each case, though there was a pine dominant phase, spruce pollen was present in abundance. In bogs of more southern latitudes *Picea* pollen seemed to be less abundant. The writer feels that greatest attention should be given to those bogs occurring in the same approximate latitude as the Shipshaw bed from the point of view of com-

parison. In any case it may be stated that due to the paucity of *Picea* pollen in the Shipshaw peat, the flora of this bed is not typically post-glacial.

The possibility remains that the Shipshaw peat is interglacial. The fact that it is a buried deposit at relatively high altitude (Legget, 1945) lends support to this view.

In seeking evidence to support or refute this interpretation from correlations of the Shipshaw flora with flora representative of interglacial stages, two major difficulties arise. One is that pollen analyses of interglacial peats are not plentiful, the other is that the few analyses that are available have been made on peats occurring hundreds of miles away from the location of the Shipshaw peat. The latter makes it difficult to draw significant or even valid conclusions from the comparisons.

Lane (1941), in his studies of interglacial microfossils of Iowa, deals with peats of the Aftonian (first interglacial) and Sangamon. In all spectra *Picea* and *Pinus* are well represented. Voss (1933), in his work on interglacial peats of Illinois, shows that *Picea* pollen outnumbers the others. The peats from which these results were obtained are Late Sangamon and Peorian in age. Forests of the Yarmouth and Sangamon interglacial stages were also determined by Voss (1939) from other Illinois peats. Of the latter, the Laura and Canton peats both show a pine dominant flora not unlike the condition in the Shipshaw peat, and they are Sangamon in age. In the Yarmouth peats on the other hand, *Picea* pollen is more numerous at most levels than *Pinus* pollen.

Tentatively the writer is inclined to a conclusion that the Shipshaw peat is interglacial and possibly Sangamon in age. That it is unlikely to be postglacial is supported by evidence given earlier (p. 139). That it may be Sangamon in age is based chiefly on the favourable comparison with the Laura and Canton peats. However, the significance of this comparison must not be overstressed as the evidence is too incomplete.

Then too there is evidence for an alternative, more speculative view that the Shipshaw peat is Yarmouth in age. Coleman (1941) recorded that the interglacial forest of the Toronto formation which he regards as probably Yarmouth, indicates a climate of four or five degrees warmer than at present, and it is a higher temperature of this order that would be more conducive to success for the Shipshaw peat flora.

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EXPLANATION OF PLATES

(All photos copied from originals supplied through Aluminum Company of Canada, Ltd.)

PLATE I

(Photos in Plate I also used by Legget (1945))

FIGURE 1.—Site of Dam No. 5. Excavation shows exposed peat face, the covering silt plain and, in the background, a spruce predominant forest.

FIGURE 2.—Site of Dam No. 5, showing exposed peat face in associated silt.

PLATE II

FIGURE 1.—Large sample of peat showing macroscopic consistency.

PLATE I



FIGURE 1. (above)

FIGURE 2. (below)

PLATE II



FIGURE 1.

"POLYEMBRYONY"
SEXUAL AND ASEXUAL EMBRYO INITIATION AND FOOD
SUPPLY

By R. B. THOMSON, F.R.S.C.

WHILE this article is primarily concerned with sexual and asexual provision for polyembryony in plants, a certain amount of comparison will be made with that in animals in order to see if there is not some common factor associated with the processes involved, particularly in their relationship to food supply. In any attempt at comparison, however, it must be realized that the terminology used in plants is different from that in animals and that, in both, the terms embryo and polyembryony are used rather loosely and in various ways. While this is confusing, it does not alter the fact that the two usual ways in which provision for polyembryony is made in plants are the same as in animals, even if they do produce much fewer adult embryos in the former than in the latter. In both, these two ways are dependent on two different modes of ontogeny of fertilized egg cells. In the more common provision, several zygotes can each undergo simple ontogeny or embryogeny (one embryo from one zygote) and produce a group of embryos of fraternal type. In the less common, one or more zygotes by undergoing budding¹ at an early stage in ontogeny can each initiate asexually or vegetatively several, sometimes many, embryos of identical type, one or more fraternal groups of such embryos being possible. There is also much similarity between the various forms of budding ontogeny in plants and animals, particularly in gymnosperms and parasitic insects where the similarity of the variety of forms is greatest. While these features suggest that some common interpretation of the origin of budding ontogeny and its relationship to simple should be possible in plants and animals, the prevalent interpretation in each is different.

In plants the explanation which has received most attention was proposed by Professor Buchholz in 1918, and has since been supported by many lines of argument and its application extended in numerous publications by himself and his students. It would also seem that this explanation has been widely accepted, if one can judge by the little

¹Although several other terms are in current use for this provision, such as one-egg twinning, fission and cleavage, the writer prefers that used by Stockard, "budding," because it suggests the vital character of the vegetative method of embryo initiation involved in this type of provision for polyembryony.

criticism which it has so far elicited. According to this explanation, the budding form of ontogeny in the pine, which provides for the asexual initiation of four embryos by each zygote, is the most primitive in conifers, and the base of an extensive phylogenetic series not only of earlier and later forms of budding ontogeny but of simple as well. On the other hand, investigators of animal embryogeny generally hold that the various forms of budding ontogeny have originated independently from simple not only in species belonging to distantly related alliances but in some cases in those belonging to closely related families.

These different viewpoints are dependent on fundamental differences in the type of evidence on which they are based. The Buchholz interpretation is based on the various forms of budding ontogeny being invariable under different conditions to such an extent that on their morphological features they can be arranged in phylogenetic series. On the other hand, animal embryologists in general (particularly experimentalists) regard all forms of budding ontogeny as inherent or constitutional potentialities which are brought into expression as conditioned responses so that any phylogenetic significance which may be associated with the process must be sought not in budding itself but in the hereditability of the conditions necessary to bring it into expression.

The amount of evidence supporting the bases for these opposite viewpoints is very different. Investigators of budding in animals have given much attention to experimental work and by it have demonstrated that asexual initiation by budding ontogeny is a latent feature of many species which normally reproduce by simple embryogeny. They have also determined experimentally that this potentiality may be eliminated by specialization. While but little such experimental work has been done with plants, possibly because of greater difficulties due to enclosure by tissue of the early stages in ontogeny, what little has been done is at least suggestive that budding ontogeny is also a latent feature in plants. On the other hand no experimental work has been done by Buchholz in support of his view that various forms of embryogeny are so constant under different conditions as to have phylogenetic significance, nor has he referred to any such supporting work by other investigators. Because of this, if there is a sound basis for the occurrence and latency of the potentiality for asexual embryo initiation by budding ontogeny in plants as well as animals, it is clear that the interpretation of budding in animals is more likely to be applicable to both than that proposed by Professor Buchholz in conifers.

In the writer's opinion there is such a basis and one which is not only common to plants and animals but is also present in other forms of asexual embryo initiation such as that by individual gametes in both plants and animals, and in the former by other cells of the female gametophyte, by cells of the endosperm, nucellus, integument, etc. This basis is clearly indicated by the sequence of evolutionary advance involved in unicellular and multicellular organization. While the outline of this sequence, which follows, does not imply that the higher plants and animals with which we are concerned originated from any living unicellular organism, the dependence of their reproductive and somatic processes on uninucleate cells does imply that the uninucleate cellular condition had been attained before these higher forms originated. Thus if, as some claim, a free nuclear condition originally preceded uninucleate cellular organization, this could only mean that any occurrence of a free-nuclear condition in these higher forms might have to be regarded as reversionary or atavistic in character.

In unicellular organisms the vegetative and reproductive functions are combined. This is also true of temporary multicellular organisms. Here the cells are loosely held together and easily separate, each cell being able to reproduce a type of organism similar to the original. Permanent multicellular organization is, however, incompatible with the retention of the two functions by the same cell, and while a safe limit of reproductive cells is all that is necessary to ensure the perpetuation of the organism, there is no such limit necessary in the case of those assigned to development of the soma (body or vegetative part of the organism) unless such development should interfere with the reproductive cells. This it never does but, on the contrary, ultimately provides completely for their nourishment. In the early stage of permanent multicellular organization, when reproduction is asexual, control over it is attained by setting aside certain cells especially for reproduction. While this function is confined to these cells under normal conditions, the cells of the soma retain to a great extent the potentiality to reproduce the whole organism, and although this potentiality is usually held under control (latent), it can express itself under some modification in the conditions under which the reproductive and somatic cells function normally. Asexual reproduction is, however, a slow method of attaining advance in organization when compared with sexual, which not only provides facilities for combining advances in different forms but also, in the sensitiveness of the meiotic process by which its gametes are formed, provides perhaps even greater facilities for such advance, affording as it does an important basis for the natural or artificial production of polyploid forms of different types.

In sexual reproduction the individual gametes retain the potentiality to reproduce the whole organism from the asexual reproductive cells from which they are differentiated and although they function normally by fusion and producing an embryo by simple ontogeny, thus permitting the full benefits of the amphimictic process to be realized, they can, under exceptional conditions, revert to asexual embryo formation (androgenesis and parthenogenesis). From the gametes, individual cells of the soma derive their potentiality to reproduce the whole organism and retain it until eliminated by specialization. Since the early formed cells of the soma are less specialized than the later, the reason for the frequent retention by them of this potentiality is evident. When present, however, after the establishment of sexually initiated simple embryogeny (one embryo from one zygote), it can express itself only under some modification of the conditions under which simple embryogeny normally takes place, as demonstrated by the extensive work on animals. Thus the potentiality to reproduce the whole organism can be regarded as an inherent or constitutional feature derived from the original organization of the cell, and one which if not lost by specialization, or if not kept under control, interferes with evolutionary advance in multicellular organization of the soma.

Since this basis is common to plants and animals, and since it does not permit phylogenetic significance being attached directly to different forms of budding embryogeny, it is obviously opposed to the explanation proposed by Professor Buchholz. Because he has not taken this basis and its implications into account, the first part of this article will be devoted to the consideration of his explanation in order to see if it justifies the phylogenetic deductions drawn from it. This will require rather long discussion because of the variety of arguments advanced in the many important contributions which he has made to our knowledge of conifer embryogeny during the period since 1918 when his interpretation was first enunciated. Moreover, the writer considers that pertinent evidence has been omitted, and, although its inclusion will prolong the discussion, that it is desirable to incorporate it in this part in order not to interfere with the presentation in the second part of experimental and other evidence of relationships between food supply and different forms of embryo initiation and development which the writer considers it necessary to evaluate before assigning phylogenetic significance to any of them.

PART I

THE BUCHHOLZ EXPLANATION

This explanation, which is essentially a defence of the primitiveness of the embryogeny of the pine and of its phylogenetic significance, will be considered, first from the standpoint of its general features and their implications, and afterward from that of the particular arguments advanced. This will permit omitted evidence of a general character to be disposed of before considering that involved in particular points. In both cases its inclusion will not only indicate the difficulties involved in the Buchholz explanation but also something of the line along which their solution will be suggested in Part II.

A. GENERAL ASPECTS

The original statement of the phylogenetic basis on which the Buchholz explanation rests is stated in the following quotation from his article on the "Suspensor and Early Embryo of *Pinus*" (1918, p. 216): "Polyembryony by cleavage from 1 egg is no doubt a primitive gymnosperm character, even though it has persisted to the *Ephedra* level, where it is on its way to elimination. No angiosperm has shown this form of polyembryony, which is further proof that it is a primitive character." In this quotation two features of terminology require consideration. In the first place, since budding of one or more sexually initiated proembryos is the only way that provision is ever made for the initiation of embryos asexually in the gymnosperm ovule,² and since a truly polyembryonic condition is only very exceptionally attained in gymnosperms, it is evident that the words "Polyembryony by cleavage from 1 egg" must refer to provision for polyembryony by budding embryogeny of a zygote. In the second place, this provision is referred to as "cleavage polyembryony" whether there is only one zygote present and involved or whether there are several. By the inclusion of both under the same term, however, no distinction is made between their relative values for improvement of embryogeny by embryonic selection, and yet Buchholz (1922) has drawn attention to the importance of competition between developing embryos in the case of provision for polyembryony by simple embryogeny. Aside from terminology, however, there are two difficulties involved in acceptance of the phylogenetic basis proposed in the above quotation.

The first has to do with the relative frequency of the provision for simple and budding embryogeny in lower and higher gymnosperms.

²Sedgwick summarized the evidence in 1924 and none has been presented since.

In cycads and *Ginkgo*, provision for simple (one embryo from one zygote) is the ordinary method and budding very exceptional; in conifers they are more or less equal, and in Gnetaceae provision for budding occurs in two of its three genera. Thus, in gymnosperms, if the sequence of the relative frequencies of these two provisions is indicative of the course of evolution, it is clearly that for simple which is being replaced by that for budding.

The second difficulty is concerned with the denial in 1918 of the occurrence of "cleavage polyembryony" in angiosperms as evidence that it is "no doubt a primitive gymnosperm character," and the statement made in 1926 (p. 69) that it occurs rarely in angiosperms and likely originated independently. If, however, it originated independently in angiosperms this could lend no support to the view that it is "a primitive gymnosperm character." On the contrary, the assignment of independent origin to it in angiosperms, where it occurs erratically in families belonging to different orders, is at least suggestive that it may have originated independently in the various families of gymnosperms, in which case the interpretation of its occurrence would conform with that proposed in animals and could not have the phylogenetic significance assigned to it by Buchholz. Again, while no reason is given for the 1918 denial of its occurrence in angiosperms, it may be that it is the same as that which is apparently suggested for the exclusion of animals and *Ginkgo* in the statement closely following that in which the denial occurs: "Although no matured twins have been found to arise by the cleavage of the egg in *Pinus*, this has been demonstrated for *Ginkgo* by Lyon (26). Here we have a close parallel to the animal twins which are formed by cleavage, and Lyon has shown that the twin embryos may originate from the same archegonium, remain organically connected, and develop equally to the maturity of the seed." Thus, since Buchholz gives no other reason for the exclusion of animals and *Ginkgo*, it would seem that the maturing of more than one identical type embryo might be his reason for the exclusion not only of *Ginkgo* and animals but of angiosperms as well, since maturing of identical progeny is common in animals and is much more frequent in proportion to provision for it in angiosperms than in gymnosperms. However, even if this feature or any other is intended to be the basis for the exclusion of all three, valid reasons would be necessary in order to substantiate their exclusion. Since none of any kind is given, it is not clear why Buchholz makes comparison of budding in animals with that of *Ginkgo* without including both in his explanation of the similar process in other plants. On the other hand, it is clear that it is only by elimination from his explanation of both *simple* and *budding embryo-*

geny in *Ginkgo* and in cycads (Buchholz, 1922, p. 254) that there is any justification for singling out budding in the pine for the distinction of being a "primitive gymnosperm character."

Acceptance of budding in the pine as of this character naturally required some explanation of the origin of simple in other forms. In this connection Buchholz holds that there are in plants two types of simple embryogeny concerned in what he calls "simple polyembryony." Although he includes both in this category, one is regarded as primitive, namely that characteristic of free sporing embryophytes, and the other as a derivative or specialized type, that found in conifers. In these, Buchholz considers that their simple embryogeny has been attained from "cleavage polyembryony" by a process to which he generally refers as fusion, but sometimes as elimination. This process is considered to have occurred first in the Abietineae from budding in the pine and later in other conifer families from forms of budding attained from that of the pine, the first stage in such attainment being that of *Sciadopitys*.

Before giving consideration to the basis on which Buchholz decided in favour of the fusion idea, it may be pointed out that, while his application of it to plants is new, it is very old in animals. It originated in the "concrecence" theory of the origin of bilateral symmetry and involves fusion of the potentiality to form two complete individuals from one fertilized egg. While this theory received some support from "Spermatists" of an old school who attempted to explain it on the assumption that two antherozoids were involved, its main support came from the interpretation of the external morphology of monstrosities such as Siamese twins which, because of their more spectacular character in animals, have attracted more attention than in plants. Later detailed study of the origin of their tissue connections, however, revealed that while a small amount of continuity of tissue was attained in some cases at late stages in ontogeny, by far the greater proportion was present at early stages. From this it was concluded that animal monstrosities should be interpreted as the result of incomplete separation of asexually initiated embryos originating by budding ontogeny of a zygote.

On the other hand, although Buchholz makes reference to a close parallel between twinning in animals and *Ginkgo*, and particularly to organic connections in both, he does not indicate that any tissue connections have been made subsequent to budding. Thus there is lacking an important element of proof that simple embryogeny in conifers is of the fusion type and therefore different in origin and character from the simple in other plants.

Even with such evidence lacking it is of interest to note how Buchholz attempts to lessen the disadvantages entailed in the necessity for conifers to have undergone this roundabout and inefficient way of attaining advance in the organization of their fusion type of simple embryogeny, when the free sporing forms from which he derives their embryogeny have attained an even greater degree of advance by their simple (non-fusion) type of embryogeny and with less diversion or waste of food resources of the mother plant. From the beginning of his work he seems to have had an idea that the greater elimination of developing embryos involved in budding embryogeny over that in simple is of some advantage. Thus in 1918 (p. 216) he refers to "cleavage polembryony" as a wonderfully efficient way of elimination of unfit embryos and cites in illustration the possibility of thirty-two embryos being formed by budding in the pine when four archegonia are fertilized. This means that if embryogeny were simple only four embryos could be formed, and, in case only one survived, that over ten times as many of the former as of the latter would be eliminated (thirty-one instead of three). While this is certainly a good illustration of difference in elimination in the two types, it is not until much later that he (1929, p. 365) indicates the basis on which he considers that elimination in "cleavage polyembryony" is of advantage to survival of the fittest.³ Here he refers to the "best fraction" produced by one zygote being an important factor. In order to be of any permanent value this fraction must be an hereditary variation (mutation). He, however, presents no evidence that it is of such character and not merely the fraction most favoured by nutritional conditions. Even granting that the dominating fraction is of mutational origin and hereditary this would not, in his opinion, put budding on a footing of equality with simple since he still holds it inferior to simple when competing on terms of equality with the latter as indicated by the special conditions required for its survival (see discussion under Particular Aspects, 2c).

Before considering the special arguments by which he supports the primitiveness of budding in the pine as the basis for the phylogenetic interpretation of budding and simple in other conifers, it is important

³The claim made by Buchholz of the wonderful efficiency of "cleavage polyembryony" in eliminating unfit embryos is referred to by Goebel (*Organographie der Pflanzen*, 1932, Vol. 3, p. 1819) in a foot-note: "Es ist aber ganz unbewiesen, dass die zugrundegehenden Embryonen, "unfit" sind, nur weil sie langsamer wachsen als der erfolgreiche. Und untaugliche Embryonen hervorzubringen, nur um sie schliesslich zugrunde gehen zu lassen, ist ein Vorang, dessen "Zweckmässigkeit" ich nicht einzusehen vermag. Ebenso wenig kann ich die Polyembryonie für primitiv halten."

to know what led to acceptance of this basis. This presents a difficulty because Buchholz nowhere definitely states his reason. He does, however, suggest the reason by his references (1920a, p. 163; 1920b, p. 130) to certain anatomical evidence. The source of this evidence is not indicated but apparently accepted as so obviously proof that no reference is made to any of the opposing evidence then available (e.g., Penhallow, 1907; Thomson, 1912; Burlingame, 1915) which placed this view at least in question. Nor has reference since been made to it or to subsequent evidence (e.g., Thomson and Sifton, 1925; Thomson, 1940) which has made the primitiveness of the pine still more questionable. It is the almost incidental insertion of such unproven collateral evidence that makes it difficult at times to determine whether it is on embryological or collateral evidence or on both that Buchholz bases his interpretation of the phylogeny of conifer embryology. Had he clearly stated at the beginning what view of the general phylogeny of the conifers he accepts and on what grounds he does so, his phylogenetic argument would have been much more easily followed. However, by piecing together various interpretations and statements, it finally became evident that the basic consideration leading to acceptance of the budding embryogeny of the pine as the most primitive conifer type rests on acceptance of the view of the Harvard school of anatomists who regard the pine as the most primitive living conifer; and that it is with this view that the embryogenies of the pine and other conifers are to a large extent required to conform. The Harvard view of the degree of primitiveness of the pine is, however, confined to conifers and acceptance of its budding embryogeny as a "primitive gymnosperm character" implies even greater primitiveness for the pine embryogeny than the Harvard school claims for its anatomy; and would therefore require more substantial defence than that for the primitiveness of the complex organization and anatomy of the vegetative organs and female cone of the pine.⁴ In any event, in so far as the writer has been able to ascertain, acceptance by Buchholz of the pine embryogeny as the most primitive in conifers is dependent on collateral evidence which not only has not been substantiated but has strong adverse evidence confronting it.

There is one other general feature which requires consideration

⁴Saxton refers to most of these specializations in a rather concise statement in an unpublished manuscript: "In almost every respect it (the pine) shows the most complex organization of any genus, in its growth phenomena, in the nature of its foliage, in the organization of its female cones, in the structure of its wood, medullary rays, ray-tracheids and resin canals, in its embryology and in its symbiotic relation with fungi, etc."

before giving attention to particular arguments advanced by Buchholz. Since quotations from his work will be used in their discussion, and since he has not defined the terms embryo and polyembryony which occur frequently in them, it is necessary, in order to avoid distraction from the points under discussion, to understand the way in which he uses these terms.

In plants, the term "proembryo" is the established one to designate stages in embryogeny up to the initiation or formation of adult organs, to which stage the term embryo is restricted. In conformity with this accepted terminology in plants, the term "polyembryony" is properly applicable in the pine or any other conifer only to the condition where one ovule produces several *mature* embryos, a condition which is rarely attained by any of them. Buchholz, however, disregards the accepted distinction between proembryo and embryo and uses the terms embryo and polyembryony in various ways.

In the case of forms reproducing normally by simple embryogeny, he applies the term embryo to various stages of proembryo development and when there are several such proembryos present he calls this condition "simple polyembryony." While this use of the terms embryo and polyembryony is misleading in that it confuses realization with provision for it, their use is more misleading in connection with the forms he includes under "cleavage polyembryony."

Here, for example, he includes free nuclei and rosette cells, to which he refers respectively as "embryo initials" and "embryo cells," and yet in neither case have they been shown to form embryos. In this connection the reference by Buchholz (1929, pp. 386-7) to the work of Jäger (1899) on *Taxus* is significant: "Jäger ('99) states, in *Taxus baccata* it seems as though every proembryonic cell has the capacity of giving rise to an embryo. He observed and figured rosette cells (not always present, and not shown in Fig. 20) and hinted at their occasional division. He almost anticipated the essentials of my interpretation that the conifer embryo is composed of many embryo initials." In the first place it may be noted that it is not after discussing rosette cells, but after referring to the formation of free nuclei and cells in detached embryonal tubes, "Embryonalschläuche," that Jäger (p. 282) makes the statement referred to: "Ich gehe noch weiter und sage, dass überhaupt alle Zellen, die aus dem Keimkern hervorgehen, das Bestreben haben, Embryonen zu bilden." The important point, however, is that "das Bestreben haben" means have the urge or endeavour, not the capacity; for, even if the quoted words do not make this clear, Jäger indicates in the same paragraph a possible way in which their *capacity* to form embryos could be proven experimentally by greater concentration of food supply,

but adds: "Leider wird dies Experiment kaum ausführbar sein." Thus instead of Jäger's work being almost anticipatory of the Buchholz explanation it really points to one of the basal defects in it, confusion of *possible* capacity with *proven* capacity, a defect which is so concealed in his application of the terms embryo and polyembryony that the importance of this distinction is not readily apparent.

In fact, it is to this point that the only definite criticism of the ideas of Buchholz which has so far appeared is directed, that of Doyle and Looby (1939, p. 140), who state: "Buchholz would seem to be obsessed with the idea that every cell of an embryonic group is an actual embryo initial. Rather are they to be considered potential embryo initials." Although Doyle and Looby in their all too brief and rather incidental criticism do not suggest any factor responsible for bringing this potentiality into expression, or how the potentiality came to be present, they do suggest comparison with budding embryogeny in animals where the effectiveness of various procedures has been demonstrated experimentally.

B. PARTICULAR ASPECTS

The great importance which Professor Buchholz attaches to particular morphological evidence makes it necessary to go into some detail in discussing it, and since it has not been assembled in any one paper, pertinent features will be brought together as far as possible by quotations from various papers. The points to be considered will include such features of the ontogeny of the embryo as (1*a*) the free nuclear condition; (1*b*) the apical cell; (1*c*) rosette cells and (1*d*) cap cells; (2*a*) the source and character of budding embryogeny; (2*b*) the origin of budding and simple embryogeny, and (2*c*) the conditions required for their survival.

1*a*. Free Nuclei: From two statements in his original paper it is evident that Buchholz (1918, p. 217) was at first undecided on the phylogenetic significance of the free nuclear condition: "In *Sciadopitys* Lawson found eight free nuclei before organization into tiers takes place. This is very significant, for here we may have this extra free nuclear division result in more embryo initials, a thing which should bring about a greater display of cleavage polyembryony than in *Pinus*," and "The writer believes that the embryo of *Sciadopitys* is more primitive than that of *Pinus*." It was thus apparently with some misgiving that he decided that the more extensive free nuclear stage in *Sciadopitys*, which may continue to the fifth or sixth nuclear division (Tahara, 1937), and its more extensive and later

budding embryogeny were indicative of greater specialization than the less extensive and earlier development of the corresponding features in *Pinus*.

In so deciding, Buchholz makes no reference to the earlier interpretation of the free nuclear condition advanced by Coulter and Chamberlain (1903), who, after a study of its extent in cycads, *Ginkgo*, and conifers, concluded that the less extensive in *Pinus* was the more specialized condition. Later, however, Buchholz (1931b) reversed his original decision, putting it into line with the Coulter and Chamberlain interpretation. This reversal is shown by his derivation of the less extensive free nuclear condition in the embryogeny of *Callitris*, *Actinostrobus*, etc., and its absence in *Sequoia sempervirens* from the more extensive in *Sciadopitys*. Recently Buchholz (1941, p. 33) has extended the reversal to all Podocarpaceae, and gives first place to the extent of free nuclei as a criterion for determining the phylogenetic relationships of their embryogeny: "So far as embryogeny is concerned, these relationships are shown by the number of free nuclei before walls appear (whether 32, 16, 8, 4 or 2). . . , etc." No evidence in justification of these reversed interpretations has been offered and yet much evidence would be required to establish their validity and even the possibility that free nuclei have the phylogenetic significance assigned to them throughout his work.

In contrast to the importance which Buchholz places on the phylogenetic interpretation of free nuclei is the little attention that he gives to the cause of free nuclei in the pine or any living conifer. In this connection he rather incidentally refers to and accepts the suggestion made by Chamberlain (1910, p. 422; 1935, p. 430) which ascribes the cause to inability of the nucleus of the zygote to segment such a large mass of cytoplasm as that in most gymnosperm eggs. This suggested cause, however, did not prevent Chamberlain from also reversing the previous interpretation by Coulter and himself (1903) of the less extent of free nuclei in the pine as more specialized than the greater extent in certain other conifers and in cycads and *Ginkgo*. This reversal and the reason for it are stated by Chamberlain (1910, p. 423) as follows: "While it might be tempting to regard forms with a large number of free nuclei as primitive, it seems probable that number of free nuclei is correlated merely with the size of the egg. That the number does not indicate antiquity is shown by the fact that *Dioon* has 1,000 free nuclei while *Pinus* has only 8 . . . , and yet *Pinus* is a much more ancient genus." In this connection it is interesting to note that, although for a considerable time after his *Dioon* work Chamberlain was impressed by the great primitiveness of the pine, he later (1935, p. 144)

came to the conclusion that the simple form of embryogeny in cycads and *Ginkgo* was the most primitive in gymnosperms. While this implies that a greater number of free nuclei is more primitive than a smaller, he did not indicate the phylogenetic significance of this in connection with the fewer free nuclei in the proembryo of the pine.

From the above discussion it is clear that there must be something wrong with the suggested explanation of the cause of free nuclei proposed by Chamberlain and accepted by Buchholz since it involves so many reversed interpretations of their number. That there is a certain amount of correlation between egg size and number of free nuclei is undoubtedly true although neither Chamberlain nor Buchholz has presented the obvious kind of evidence (actual measurements) required to prove that there is. Had they done so, however, it should have revealed to them that a source of food supply external to that of the egg is involved in and important to attainment of the free nuclear condition. Even without actual measurement of egg size in gymnosperms, the importance of an external supply should have been apparent had the conditions under which the free nuclear stage occurs in the development of the female prothallium in the gynospore of seed plants been compared with those under which free nuclei occur in the proembryo. In the gymnosperms in which the number of free nuclei is greatest, the egg is so large that it can be seen with the naked eye, particularly easily in stained sections, whereas the gynospore, even when stained, is so small that it requires a microscope to distinguish it from the surrounding small cells. The gynospore, having no mass of food material in it, must get the supply for its extensive free nuclear stage from outside, an abundant source being provided by the tapetum and surrounding cells. The causal factor therefore cannot be inability on the part of nuclei to segment a large mass of cytoplasm as claimed by Chamberlain, but must lie in some more vital activity of an excess supply than that of its mass acting in a negative or inhibitory capacity, a point to which further reference will be made in Part II. The importance of an external supply, however, was not realized by Chamberlain, probably because he has always held, as indicated in his latest book (1935, p. 430), to the old idea that the gynospore in seed plants was large, a macro- or megaspore, whereas it is small, sometimes smaller than the androspore (Thomson,⁵ 1927).

⁵The use of the terms macrospace or megaspore and microspore is still prevalent in the literature of seed plants today, nearly twenty years after their spores were shown by actual measurements to be homosporous. Whatever the reason for such conservatism, the continued use of these terms perpetuates an error which is just as absurd as it would be if these terms were applied to the spores of such a form as

1b. *Apical Cell*: The significance attached by Buchholz to the presence of an apical cell and its duration in the ontogeny of the embryo of the pine is indicated in his 1918 paper (p. 214): "It is evident that in *Pinus* a primitive condition is found, in which the apical cell is still functional for a considerable period." This was demonstrated by Buchholz up to the 800- and found usual to the 500-cell stage, a duration not found to be exceeded in any other conifer. The inference drawn from its presence and prolonged persistence in the pine is stated on page 219: "Thus it looks as though nearly all the embryos of the Coniferales may be derived from an ancestor with cleavage polyembryony and an apical cell like *Pinus*, differentiating into several more or less distinct lines of specialization. This is strong argument in support of the theory that *Pinus* is a very primitive and ancient genus."

No reference to the basis on which the above phylogenetic inferences depend is made by Buchholz in the text of his 1918 paper, but one is found in the summary (p. 221): "The apical cell represents a primitive fern character which is recapitulated in the embryogeny of *Pinus*"; and another in a later paper (1920a, p. 162): "The apical cell is present in the adult ferns and in the first stages of the pine embryo; it is absent in all adult gymnosperms and likewise in angiosperms." That it is the apical cell of the adult *Marattious* fern to which Buchholz particularly refers is evident from his use of a derivative of this group to illustrate the source of "cleavage polyembryony" in the pine (1929, p. 367). He, however, neglected to take into consideration the extensive investigations on apical cell growth by Bower (1908), who demonstrated that, when a single apical cell is present in the embryo of *Marattious* ferns, it is replaced in adult organs by a meristem of several cells. The argument for the primitiveness of the apical cell in the pine as based by Buchholz on the presence of an apical cell in the adult *Marattious* fern is therefore directly opposed by the work of Bower. Even on the basis of the occurrence of an apical cell in the young sporophyte of this group, or in the young or adult of any fern or other vascular cryptogam it would still run counter to the conclusion of Bower (1908, p. 678), who states that "the more definite segmentation with a single initial is a derivative state in the sporophyte, and that

Equisetum because of their heterothallic development. The result is regrettable not only as suggested in the case of the cause of the free nuclear condition, but in that it diverts attention from the important differences in mode of provision of nutrition for the spores of free sporing and seed-habit plants, differences the recognition of which is of vital importance to the proper interpretation of the factors involved in the origin of the seed habit.

with several initials the more primitive," a view which he elaborated in 1935 as indicated in his conclusion (p. 326): "The Eusporangiate state, with its more complex but less precise cleavages, was the prior type."

Although Buchholz has used the apical cell argument throughout his work, he evidently did not find its basis completely satisfactory since in 1926 (p. 62), he refers to the association of apical cell and cleavage polyembryony as "not positive proof" but as "suggestive of the primitive nature of the condition of splitting embryos." This may have been what led him later (1931a, p. 122) to seek further evidence by comparing the ontogeny of the apical cell in the pine embryo and in the prothallial development of the leptosporangiate homosporous fern. In the pine, Buchholz (1918, p. 201) considers that four apical cells originate at the first formation of walls in the proembryo, that the first division of each, which is transverse, gives rise to a suspensor cell, and that the succeeding divisions, which organize the undifferentiated massive stage of the embryo, are oblique at first but soon establish an apical cell with three cutting faces. While it is clear that such ontogeny by an apical cell in the pine is practically identical with that of the filamentous prothallial development of homosporous leptosporangiate ferns, it is well known that the embryo development in leptosporangiate ferns, whether homosporous or heterosporous, has no filamentous stage but is massive from the beginning.

It is therefore clear that before concluding that the ontogeny of the apical cell in the pine is primitive and "suggestive of the primitive nature of the condition of splitting embryos," Buchholz should have determined that it was not just an accompaniment with modifications, of apical filamentous growth becoming massive wherever it occurs. Thus, as is well known, there is apical cell growth similar to that of the pine embryo, though of more limited extent, in the development of the sporangium of leptosporangiate ferns but not in that of the cusporangiate. Illustrations of capitate (glandular) hairs developing on angiosperm leaves and undergoing a similar ontogeny may also be found in ordinary botanical text-books and wall charts. Moreover, a closely parallel condition obtains in the embryo of certain angiosperms, although usually with a less extensive filamentous stage. Here, while growth in length is supplemented by division of intermediate cells, divisions are usually more abundant toward the apical cell from which the undifferentiated massive stage develops by one to several individual three-cutting-face cells. With regard to filamentous growth at the opposite pole of the angiosperm embryo, a statement by Strasburger (1878, p. 665) on the apical cell growth of the filamentous pro-

embryo into the micropyle in *Gymnadenia* (where there is much fluid food) is so pertinent that it may be quoted: "Er zeigt eine selbständige Entwicklung und vermehrt die Zahl seiner Elemente durch Theilung der terminalen Zelle."

On the other hand, in the embryo development of cycads and *Ginkgo* there is no filamentous stage, but after extensive free nuclear and cellular divisions a "meristem" of several cells continues the process. The ontogeny of the embryo in the cycads should be all the more significant since these plants are recognized as closely related to the eusporangiate ferns by the classic work which led to the establishment of the Cycadofilices and later to that of the Pteridospermeae, and it is the eusporangiate fern type that Buchholz uses in illustrating the ancestor of the pine. Moreover, since the cycads have very valid claims (hausterial pollen tube, zoidogamous fertilization, etc.) to being primitive gymnosperms near the "early advent of siphonogamy," at which time Buchholz (1926, p. 61) considers that cleavage originated as a "primitive gymnosperm character," the significance of a meristem in their embryo ontogeny should be all the greater.

Even granting that the basis of the apical cell argument as originally proposed by Buchholz is sound, i.e. that an apical cell is a primitive feature of fern embryogeny, he does not explain why its association with budding embryogeny in the conifers should be indicative of greater primitiveness than its association with simple embryogeny. Since this possibility has not been eliminated and since valid reasons would be necessary to support the view that the derived association was carried over into the Abietineae, and not the ancestral association with simple embryogeny, it can only be inferred that any primitiveness which has been ascribed to the relationship of apical cell and budding embryogeny, instead of being entailed in the apical cell itself, is dependent on acceptance of the pine embryogeny as the most primitive conifer type. This inference would seem to have been at least partially realized by Buchholz (1920a, p. 162) himself in making the following statement: "To assume that cleavage polyembryony is a derived feature would take away all phylogenetic significance from this structure (apical cell)."

Finally, in Abietinae, Buchholz (1920a, p. 162) supports his argument for the primitiveness of budding embryogeny in the pine on the basis of absence of an apical cell in other Abietinean forms with simple embryogeny (*Larix* included). Schopf (1937, 1943), however, has reported that there are four apical cells in the early stages of the simple embryo of *Larix*. Here he considers that at the 16-cell stage there are four "polarity units," each with an apical cell and a suspensor cell as in

Pinus, and that three of these eventually abort leaving a single unit in which he says apical cell growth is eliminated usually before the embryo attains the 100-cell stage. Schopf is, however, undecided on the phylogenetic significance of the duration of the apical cell in view of the present state of knowledge of its duration in ancestral forms. On the other hand he makes the primitiveness of his polarity units depend on filamentous growth in certain free sporing embryophytes being primitive. In this connection he (1943, p. 70) attaches considerable importance to the "primitive spindle" theory of Bower but does not discuss the possibility that such elongated growth of the proembryo has been evolved as a secondary feature associated with the acquirement of better nutritional conditions for the development of the embryo proper, as suggested by the work of Land (1923). This is a point, however, to which further attention will be given in Part II since it involves much flexibility of embryogeny which is important in understanding other features of embryogeny. Moreover, Schopf does not explain why four polarity units should be involved and indicative of primitiveness in Abietineae when there is only one in free sporing forms. Without considering these difficulties, however, he interprets his results in *Larix* as indicative of "delayed cleavage polyembryony" resulting in simple embryogeny, and considers that a similar interpretation will be found to obtain in other Abietinean forms with simple embryogeny. This interpretation, however, is based on acceptance of budding in the pine being more primitive than simple in *Larix*. On the other hand it is evident that the conditions described in *Larix* could equally well be interpreted in the reverse way, i.e. as evidence of an incipient stage in the attainment of the type of budding ontogeny in the pine; and Schopf gives no reason for not so regarding it.

Thus, when the various apical cell arguments are critically examined, they fail to produce any evidence of "the primitive nature of the condition of splitting embryos," and consequently cannot be regarded as a "strong argument in support of the theory that, *Pinus* is a very primitive and ancient genus." On the other hand their examination does indicate the importance of a broad consideration of all conditions involved, particularly those of an ecological or physiological nature, before attaching phylogenetic significance to a feature which is so erratic in distribution as growth by an apical cell.

1c. *Rosette Cells*: The cells of the rosette plate and the proliferations of these which Buchholz calls "rosette embryos" were originally regarded by him as of greater significance to his interpretation of "cleavage polyembryony" than either free nuclei or apical

cells. One of his arguments is that "rosette embryos" have apical cell growth, at least in the family of conifers which he accepts as the most primitive, the Abietineae. Since as already shown an apical cell may occur in any filamentous growth, particularly in those becoming massive in the region of such growth, it is unnecessary to deal further with this point.

His other arguments (1926, pp. 62-63) are concerned with the conclusion that rosette proliferations in the conifers are "the result of an extreme expression of cleavage polyembryony" and represent "survivals from a condition of greater cleavage polyembryony." In connection with this conclusion there is a statement in his 1918 paper (p. 205) which is of interest. This is made after referring to variations in development of rosette cells: "Rosette embryos develop less rapidly than the primary embryos, abort at an early stage, and it is entirely outside the range of possibility that they may ever contribute the embryo of the seed." Thus the conclusion with respect to "polyembryony" and rosette proliferations is open to even more fundamental criticism than that already indicated in connection with the interpretation of the work of Jäger, and that expressed by Doyle and Looby. Here it is based on a definite statement that it is not necessary to have proof that rosette proliferations in conifers can produce embryos in order to conclude that "cleavage polyembryony" was present in their ancestry. The defect in this conclusion becomes even more apparent when it is realized that proliferated growths, which occur frequently in the embryogeny of both higher and lower embryophytes, could be similarly interpreted and thus invalidate the basis on which Buchholz has concluded that "cleavage polyembryony" in the pine is "a primitive gymnosperm character."

Even granting, however, that in case of accident to the terminal cells or from some unknown cause an embryo should be formed from a rosette cell, this would not necessarily support the view that proliferations of rosette cells represent "survivals from a condition of greater cleavage polyembryony." That it should do so would require proof that this condition was present as a constitutional feature in the ancestor of the survivors and the only suggestion made to account for this entails the postulation of its origin (see 2b) by mutation in a hypothetical form where proof of such origin is impossible.

Finally there is a difficulty in connection with the phylogenetic interpretation of the presence of rosette cells and the extent of their proliferations. This, as in the case of free nuclei, involves reversals. For example in the Abietineae their gradual elimination is regarded as accompanying the change from budding embryogeny in the pine to

simple in other Abietineae, the simple embryogeny of *Pseudotsuga* showing no evidence of rosette cells. On the other hand, *Cephalotaxus*, the embryogeny of which is regarded as having been derived from that of *Pinus* through an intermediary *Sciadopitys*-like form with less extensive rosette development than *Pinus*, has rosette structures which Buchholz (1931b, p. 260) says are "more constantly present, persist longer, and develop to an older stage than do those in *Sciadopitys*."

1d. Cap Cells: The structure which is called "Bohrspitze" by Goebel (*loc. cit.*) is referred to by Buchholz as "the cap" and assigned a very different function to that indicated by the term used by Goebel. It covers the end of the embryonic axis pene-

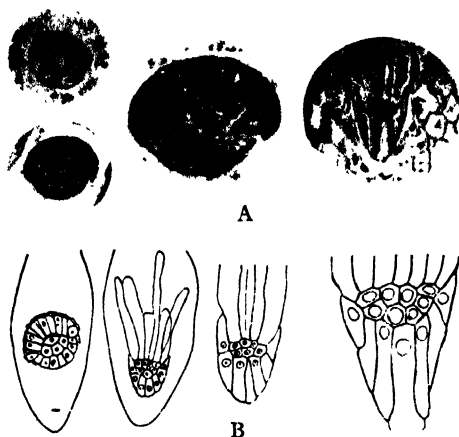


FIGURE 1.—A. *Agathis australis* (from Sharp, 1926, p. 76, fig. 21, after Eames, 1913); B. *A. australis*—three to left—(from Buchholz, 1929, p. 379, fig. 15, after Eames, 1913; *Araucaria brasiliensis*—one to right—(from Buchholz, 1929, p. 379, fig. 15, after Burlingame, 1915).

trating the bulk of the food material in the female prothallium and is regarded by Buchholz as an inhibitor of budding embryogeny in one of the several lines of phylogeny giving rise to his fusion types of simple embryogeny in conifers, that leading from the pine through *Sciadopitys* to the Araucarians. If, however, the cap does function in this way, it must do so in addition to its function as a food absorbent structure, a function which is obvious from the fact that it is through epidermal cells that nutrition for embryo development is provided. In *Agathis* and *Araucaria* (Fig. 1B, cf. *Cephalotaxus*, *Taxus*, etc.), for example, these cells develop into long absorbent tubes similar to the embryonal

tubes at the opposite pole. Moreover, a much more extensive and later developed cap occurs in cycads⁶ than in conifers, as may be seen by comparison of B and C in Fig. 2 with B of Fig. 1, the latter illustrating its greatest and latest development in conifers. The cap cells of cycads, however, do not elongate so much as those of the Araucarian type and are apparently not cast off, as in the latter, but continue as an absorbent layer when the cotyledons are forming. Buchholz, how-

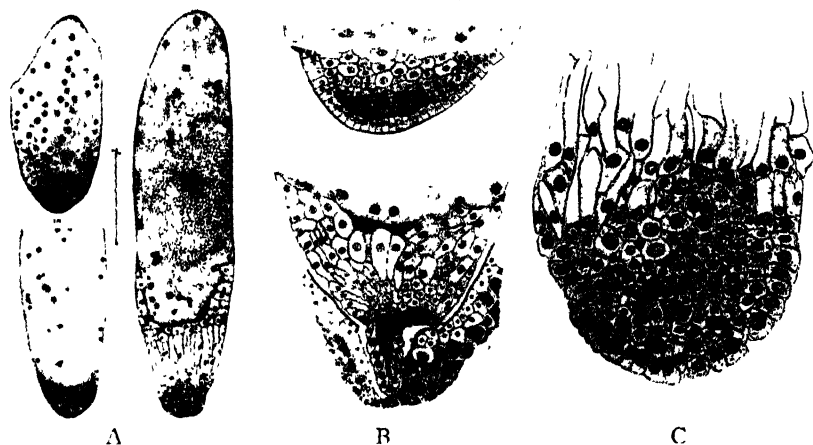


FIGURE 2.—A. *Zamia floridana* (Coulter and Chamberlain, 1903, pl. 7); B. *Stangeria paradoxa* (Chamberlain, 1916, pl. 26, figs. 22, 23); C. *Macrozamia spiralis* (Brough and Taylor, 1940, p. 517, fig. 74).

ever, has not taken the presence of a cap in these most primitive of living gymnosperms into account before assigning an inhibitory function to it in conifers. On the other hand, while the writer admits that the cap in all forms may have the additional function of inhibiting an innate potentiality for budding embryogeny, it is clear that the presence of a cap does not provide any evidence in support of the view that this potentiality found any, let alone greater, expression in the ancestry of any of them.

2a. Source and Character of Budding: Buchholz could find in neither living nor fossil forms an ancestor with suitable budding embryogeny from which that of the pine could have been inherited, and without discussing even the possibility that simple embryogeny in conifers is more primitive than budding,

⁶The later stage at which the cap cells become prominent in cycads has not been described in *Ginkgo*, but an earlier one has, and in it a cap similar to that of cycads at the corresponding stage is formed.

constructed a hypothetical fern-pine transitional form with a type of budding from which he considers that of the pine was derived. Of the source of this ancestor Buchholz (1929, p. 364) states: "Cleavage polyembryony was no doubt derived originally from the condition of simple polyembryony, for no instance is known among Pteridophytes where the zygote gives rise to several embryos. During the transition from ferns to seed plants or very soon after this time of transition, cleavage polyembryony had its origin"; and again (1929, p. 367): "If we wish to picture a hypothetical transition of embryogeny from ferns to conifers we will select a fern of the eusporangiate type as a starting point."

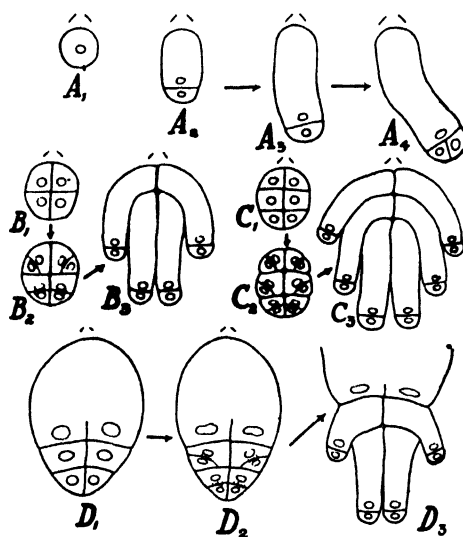


FIGURE 3.—Diagram representing hypothetical transition from ferns to conifers (Buchholz, 1929, p. 368).

Fig. 3 is a reproduction of the illustration of the series of stages which Buchholz (1929, p. 368) regards as involved in the inheritance of the embryogeny of the pine from that of the eusporangiate fern type with a suspensor as in *Danaea* and *Angiopteris*. In it A shows the normal development of the procumbent of one of the eusporangiate ferns; B and C show that of the hypothetical form derived from A (8-celled with only 4 cells shown in B, and 12-celled in C) with each cell divided into an embryonic initial and a suspensor. Of the D stages Buchholz states in the legend of the figure that "D₁ shows the condition of C₁ accompanied by an enlargement of egg; D₂, D₃ develop-

ment of embryos from the lowest tiers in D_1 with abortion of upper incompletely walled tier."

The fern (A) chosen for illustration of this starting point is thus homosporous and of eusporangiate type, in which respects it is like seed plants (Thomson, 1927). It is of interest that Buchholz (1926, p. 59) has also referred to a heterosporous leptosporangiate fern as being involved in the transition. Although he has offered no explanation of his choice of the former for illustration it seems likely that he did not consider the latter suitable because at the time he attached much phylogenetic significance to suspensorial cells in conifers and no leptosporangiate fern has a suspensor. He even constructed a rather elaborate, phylogenetic classification of these cells (prosuspensorial, primary, secondary, etc.) based on the sequence of their origin in proembryo development. In his most recent paper (1942, p. 158), however, he has come to the conclusion that there is "probably no valid reason" for making several of the distinctions in this classification because he found no difference in the morphology of the cells on which to base phylogenetic deductions.

The second stage, that of the hypothetical fern-pine transitional form, is constructed on the massive type of proembryo development (B_1 and C_1) characteristic of leptosporangiate ferns; and onto each of their 8 and 12 cells is superimposed the filamentous type of growth characteristic of the Eusporangiateae (B_3 and C_3). By all authorities on ferns, however, the Leptosporangiateae are regarded as a divergent line of development from that leading to seed plants. Moreover, the evidence on which Buchholz bases the phylogenetic sequence indicated in the figure, from provision for more embryos in C_3 to fewer in D_3 , requires consideration. This apparently rests on his interpretation of the work of Kildahl (1907), who found budding in the pine at the 8-'celled' instead of the 12-'celled' stage; at least the writer has been unable to find any reference to other evidence. According to Buchholz this 8-'celled' condition is due to the possible early collapse of one tier of free nuclei, and although neither Miss Kildahl's nor his own work affords any evidence of such collapse, he (1918, p. 215) interprets this 8-'celled' condition "as a real preliminary stage in embryogeny." Then after including all stages (A to D) in his fern-pine transitional form in the illustration in his 1929 paper, and without referring to any further evidence, he makes the following statements in the text (p. 369): "Thus we see that the transition from ferns to conifers such as the pine is not a difficult one on a theoretical basis. Of course this transition may actually have been much more complicated; it hardly seems possible for this change to have occurred on

a more simple basis than that which is here represented, except that the number of embryos first formed by cleavage might have been smaller; it may have begun with cleavage into two or four cells and become gradually more complicated with many embryo initial cells."

In the above quotation the phylogenetic sequence described makes the provision for asexual initiation of fewer embryos at an early stage in ontogeny more primitive than that for initiation of more at a later stage, and in the figure this sequence (A to C) is illustrated and another (C to D) that makes the provision for more at a later stage more primitive than that for fewer at an earlier stage. While objection to the evidence for both sequences has been put forward in the preceding paragraph, it is evident that by the use of two sequences, one the reverse of the other and yet with the same phylogenetic significance, it is possible to interpret a particular form of "cleavage polyembryony" in either sequence as primitive or specialized. For example, in the lines of specialization which Buchholz considers originate in the pine, proceed through *Sciadopitys* and then diverge to *Sequoia sempervirens* and to certain Cupressineae (*Callitris*, *Actinostrobus*, etc.), both sequences occur—that from the pine to *Sciadopitys* being of the fewer-to-more type and that from it to the others being of the more-to-fewer. By starting with his specialized forms, however, the same two types of sequence are met with before arriving at the pine, but in this case the pine would be at the specialized end of his phylogenetic lines. Thus, because each of the two sequences can be interpreted as leading to either primitiveness or specialization depending on the end selected for the starting point, it is evident that considerations other than embryological must have determined the starting point chosen by Buchholz for each.

When, however, these and other difficulties discussed previously are taken into account, it is evident that their occurrence conforms in general with the requirements of the phylogeny proposed by the Harvard school of anatomists. Whether this collateral evidence is the basis for them or not, in meeting them no consideration is given even to the possibility that variations in embryogeny may belong to the physiological plasticity category and so necessitate taking the extent of their plasticity into account before assigning phylogenetic significance to any of them. Apparently no inkling of this necessity was suggested to Buchholz, although he makes reference in the legend of the above figure to the D stages as showing enlargement of the egg and later (1931b, p. 25) suggests that "events connected with the history of the archegonial complex and resultant reduction in the

archegonial size have had something to do with the decrease in the number of free nuclei in the proembryo, the reduction in the number of embryo initials per zygote, and the number of cells in the prosuspensor."

2b. Origin of Budding and Fusion Types: The only suggestion Buchholz (1926, p. 59) makes with regard to the origin of budding embryogeny is in connection with the conditions required for its survival in his fern-pine transitional form. Here he rather incidentally indicates his view in this way: "should it have originated either as a new mutation or in a segregation following some kind of hybridization." However, since segregation following hybridization entails the origin of budding by mutation, and since Buchholz (1929, p. 387) has given no other reason for his statement that "the conifer embryo is composed of many embryo initials," and has arranged the various forms of budding in conifers phylogenetically, it is evident that all must be regarded as mutations in order to fulfil the requirements of his explanation. Many mutations would thus be involved, and while the usual breeding work required to demonstrate mutational origin of budding is of course impossible in a hypothetical ancestor, none has ever been done with either living plants or animals in substantiation of such origin.

With regard to the origin of the fusion forms of simple embryogeny which are regarded as derived from forms of budding at various stages in their evolutionary advance, Buchholz (1929, p. 366) simply suggests that a "mutational change" occurs. Here also no experimental evidence is provided that a mutational change is involved. Moreover, in neither case is their distribution accounted for on the basis of segregation, but is regarded as dependent on conditions which Buchholz considers necessary to permit of their survival.

2c. Survival Conditions: These involve a process which Buchholz calls embryonic or developmental selection as indicated in the following statement (Buchholz, 1926, pp. 58-59): "It follows from the nature of this developmental selection, that the origin of cleavage polyembryony is to be sought under conditions in which only a single egg was fertilized, or in which the fertilization of several eggs was not simultaneous or nearly so, with the result that the embryonic selection did not eliminate the smaller products of cleavage." Since both the ancestral and the fusion types of simple embryogeny are regarded as superior (dominant?) to budding when in competition with it, this competition must be either eliminated or so handicapped as to permit the inferior (recessive?) budding type to survive. Moreover,

at every stage of advance at which this budding type is regarded as giving rise in living conifers to the fusion simple type, as it is required to do to account for the presence of simple only in various families, the conditions of competition must be reversed in order to eliminate the parental budding type.

The arguments presented to establish a basis for the survival of budding are very elaborate, as are also those for the survival of the fusion type of simple embryogeny. While the writer considers that several of these arguments are not based on sound evidence, there is a point in connection with the conditions for survival of the ancestral and derived types involved in his description of the fern-pine transitional form which makes it unnecessary to discuss them. Although Buchholz has given much attention to conditions permitting survival of the derivative budding type in this form, he has not suggested anything to prevent the parental simple type from having survived and been carried over into the conifers, where it could have given rise to budding by mutation as easily as the simple of the ancestral form is supposed to have done. Moreover, if budding is inferior when in competition with simple, it would have found ideal conditions for its survival in the conifers themselves, several of which have single archegonia, e.g. *Abies balsamea* (Miyake, 1903), *A. pectinata* (Cavara, 1900), *Torreya taxifolia* (Coulter and Land, 1905) and *Phyllocladus alpinus* (Kildahl, 1908). There is also nothing in pluriarchegoniate conifers to prevent the fertilization of only one archegonium, any more than there would be in the postulated ancestor if it were pluriarchegoniate.

* * *

In concluding this discussion of the morphological basis for the interpretation of conifer embryogeny the writer wishes to point out that the use of this basis by Professor Buchholz in no way impairs the value of his outstanding contributions to our knowledge of conifer embryogeny. It does serve, however, to emphasize that the morphological expression of any hereditary feature, whether it has an old constitutional basis or is due to recent mutation, cannot be used to determine the course of phylogeny until the extent of its variability under different conditions has been determined and taken into account. In fact it was the discovery (Thomson, 1940) of association between amounts of ovular food supply and variations in anatomy of cone structure (which for over a hundred years had been interpreted on the basis of the stability of its anatomy) that directed the writer's attention to the need for investigation of certain problems of embryology associated with food supply. These will be considered in Part II.

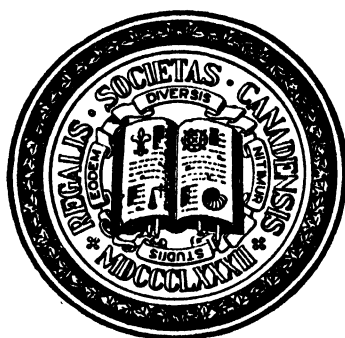
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PRESIDENTIAL ADDRESS

ANTAGONISTIC AND SYNERGISTIC PHENOMENA IN THE AUTONOMIC NERVOUS SYSTEM

By B. P. BABKIN, F.R.S.C.

ANATOMISTS in the early days knew that almost all the internal organs of the body in man and the other mammals possess a double innervation. One group of nerves, according to them, is derived from the central nervous system. These were at first called cerebrospinal nerves; they are now referred to as *parasympathetic*. They are myelinated and white and extend without any visible interruption of their course from the brain or the spinal cord to various organs, such as the heart, the abdominal viscera, and different glands. Other nerves to the internal organs, often unmyelinated and greyish-pink in colour, are derived from the ganglionated nervous system, which runs along both sides of the vertebral column. Winslow, an outstanding anatomist of the early eighteenth century, considered this nervous system to be independent of the cerebrospinal system and believed that the ganglia scattered throughout the body are its "little brains." However, he emphasized the existence of some relationship between the two nervous systems, calling the ganglionated nervous system the *sympathetic* nervous system (Winslow, 1732). "Sympathy" in the scientific language of the eighteenth century meant reciprocity. For example, certain conditions of the stomach may be reflected in the activity of the heart, and certain disturbances of the heart may affect the functions of the stomach. These "sympathies," according to Winslow, were conveyed from one organ to another through the sympathetic nervous system.

Although Winslow's designation "sympathetic" has been retained, the conception of the relation of the sympathetic nervous system to the central nervous system has changed radically since his time. We know now that the sympathetic nervous system is only a part of the whole nervous system, which has centres in the brain and in the spinal cord, its ganglia being simply the points of connection between its

preganglionic and postganglionic fibres. The whole portion of the nervous system which innervates the internal organs and which consists of the sympathetic and parasympathetic divisions has been designated the *autonomic* nervous system, distinguishing it from the *somatic* nervous system, which supplies motor nerves to the striated muscles and sensory nerves to the outer parts of the body. The course of the sympathetic and the parasympathetic fibres outside and inside of the central nervous system and their distribution to different organs has been worked out in great detail. It has been established also that the great majority of the internal organs, with the exception of the uterus, nictitating membrane, and some structures in the skin, such as the sweat glands, possess a double innervation, one nerve being derived from the sympathetic, and one from the parasympathetic division of the autonomic nervous system.

Physiological knowledge of the autonomic nervous system quite naturally could not keep pace with the anatomical data on the subject. However, there has been steady and substantial progress in this branch of physiology during the past fifty years.

One of the most difficult problems of the physiology of the autonomic nervous system is the significance of the double innervation—sympathetic and parasympathetic—of most of the internal organs.

When in 1845 the brothers Weber discovered that the vagus is the inhibitory nerve of the heart, they concluded that the sympathetic nerve must supply the heart with motor fibres. However, they were unable to prove their theory. Not until Bezold, Schmiedeberg and Ludwig, and the brothers Cyon in the years 1862 to 1875 established definitely that the heart is supplied with accelerator fibres from the sympathetic nervous system did a strong belief arise among the physiologists that the double innervation of the organs of the vegetative system is antagonistic—both excitatory and inhibitory. This was expressed very forcibly by Gaskell (1887), who wrote: "The evidence is becoming daily stronger than ever that every tissue is innervated by two sets of nerve fibres of opposite character, so that I look forward hopefully to the time when the whole nervous system shall be mapped out into two great districts, of which the function of one is katabolic, of the other anabolic to the peripheral tissue."

According to Gaskell, the motor nerves are katabolic, whereas the inhibitory nerves are anabolic. For example, stimulation of the sympathetic nerves to the heart accelerates and augments its action; this increased activity of the heart is followed by relative exhaustion of the organ—a true sign of katabolism, as Gaskell thought. On the other hand, stimulation of the vagus nerve diminishes the action

of the heart, which allows repair of its function—a symptom of anabolism.

Ten years later, in 1897, when discussing a new type of nerves—secretory-inhibitory—the existence of which he believed he had established, Pavlov (1902, p. 59) remarked: "But when it has been proven beyond doubt for several organs that the nerves which regulate them belong to two opposite groups, the same may be assumed for the glands. It is quite possible that antagonism of this nature belongs to the general principle of innervation."

Our conceptions regarding the mode of action of the nerves on the tissue innervated by them have changed substantially since Gaskell's time. Gaskell believed that katabolism excluded anabolism and that the essential processes of living matter were regulated by the two antagonistic nerves. As Barcroft (1934, p. 268) has put it in our day: "The theory of anabolic and katabolic nerves seems to have died a natural death." Nevertheless the fact remained that the nerves of the two divisions of the autonomic nervous system act antagonistically not only on the heart but on most of the internal organs, and the significance of this double innervation was and is a very real problem. The excitatory or inhibitory properties do not reside in one special group of nerves—sympathetic or parasympathetic. In some of the organs the sympathetic are inhibitory and the parasympathetic excitatory; in other organs the relations are the reverse. Or, again, both types of nerves may be supplied by one and the same division of the autonomic nervous system.

The discovery of the chemical transmission of nerve impulses did not in any way alter the conception of the antagonistic innervation of the internal organs. The nerves were considered to be cholinergic or adrenergic and the effect on the tissues of the substances liberated by them was thought to be antagonistic. Intravenous administration of acetylcholine or adrenalin was in almost all respects analogous to stimulation of the corresponding parasympathetic and sympathetic nerves. In many experiments, therefore, these substances were substituted for stimulation of the nerves.

In spite of the great amount of work that has been done in the investigation of the double innervation of different organs, this problem is still far from solved. The digestive glands like other organs are supplied with sympathetic and parasympathetic nerves. Unfortunately the data concerning the double innervation of the digestive glands are particularly confusing. Their elucidation interested me and my co-workers for many years. The following discussion is based chiefly on data obtained by us. Our facts are not presented in

the chronological order of their discovery but are cited indiscriminately, with the aim of giving a comprehensive picture of the innervation of the digestive glands. On the analogy of these glands, we hope to learn the nature of the general relationships between an organ and the two nerves which innervate it.

THE DOUBLE INNERVATION OF THE DIGESTIVE GLANDS

What does the double innervation of the digestive glands signify? On first thought this seems an easy question to answer. Like any other organ, a digestive gland consists of a number of different structural elements, namely, secretory cells, contractile tissue of muscular or epithelial origin, blood vessels. Each of these structures is innervated; some of them possess definite antagonistic innervations. For example, the muscles of the gastro-intestinal tract are supplied with excitatory and inhibitory nerves, the glandular blood vessels have their vasoconstrictors and vasodilators, and so on. The antagonistic nerves usually belong to different divisions of the autonomic nervous system. We find, however, that conditions are different when we come to the problem of the innervation of the glandular tissue itself. Are there nerves which are able to inhibit or arrest the secretory process and which are the counterpart of the nerves stimulating it?

Pavlov (1902, p. 59) believed the digestive glands to be supplied with special *secretory-inhibitory* nerves. He thought that he had proved their existence in the case of the gastric glands and of the pancreas. It was supposed that these nerves diminished the secretion of the glands by direct action on the secretory cells and not through vasoconstriction. However, in the light of new facts since discovered, the evidence on which Pavlov based his theory cannot be considered entirely convincing.

According to Pavlov, the main proof that the gastric glands were supplied with secretory-inhibitory nerves was the extremely long latent period (of one and a half hours' duration in some cases) preceding the gastric secretion elicited by faradization of the vagus nerves. Pavlov thought that the secretory-inhibitory nerves were more easily excited than the secretory nerves and therefore dominated the latter in the initial period of faradization of the vagi. We know now that the extraordinarily long latent period of gastric secretion in Pavlov's experiments was due to the removal of CO_2 from the blood by the forced artificial respiration which Pavlov applied to the animal. Browne and Vineberg (1932) in our laboratory showed that a certain CO_2 content was necessary in the blood to enable the vagi to produce a gastric secretion when stimulated by an induction current. In some

of their experiments on dogs the latent period preceding the vagal gastric secretion was only of fifteen to twenty minutes' duration. A latent period of this length under artificial stimulation of the vagi cannot be considered too prolonged, since a perfectly healthy dog with œsophagotomy and a gastric fistula starts to secrete five to ten minutes after the beginning of shamfeeding.

Recent investigations of Gesell and his associates (1942, 1945) support the data of Browne and Vineberg concerning the optimum requirement of CO_2 concentration in the blood for the transmission of impulses from the nerve endings to the tissue innervated or from one neuron to another. In a number of communications Gesell emphasized the influence of the hydrogen ion concentration of the body fluids and tissues on the effect of nerve stimulation. Here is one striking example. The duration of the respiratory after-discharge following the faradization of Hering's nerve or of the cutaneous-sensory saphenous nerve varied inversely with the duration of stimulation. This was contrary to the fact of the direct relationship of the after-discharge to the length of stimulation observed in spinal reflexes. Evidence was presented that the difference in the after-discharge in a respiratory as compared with a spinal reflex was due to the loss of CO_2 and the subsequent alkalization of the blood in the former case and the absence of this effect in the latter. Since the destructive action of cholinesterase on acetylcholine is facilitated in alkaline and impeded in acid media, the loss of CO_2 shortened the life span of each deposit of acetylcholine resulting from stimulation of the afferent nerve. This determined the rate of accumulation and the amount of acetylcholine in the nerve cells under nervous bombardment. In spite of longer stimulation of the sensory nerve less chemical transmitter was available to convey the nerve impulses to the cells of the respiratory centre than after brief stimulation.

In consequence of the work done by Anrep (1915-16), Korowitzky (1923), and Crittenden and Ivy (1937), the theory of Pavlov (1902, p. 59) that the inhibition of pancreatic secretion by stimulation of the vagus nerve was attributable merely to the effect of secretory-inhibitory fibres allegedly present in this nerve, could not be maintained. The pancreatic gland undoubtedly possesses a special mechanism, which is also under the control of the vagus and which is able to inhibit pancreatic secretion temporarily. This mechanism, however, has nothing to do with the secretory process itself but involves constriction of the pancreatic ducts by the contractile tissue which they contain and of which the vagus is the motor nerve.

Although the theory regarding the secretory-inhibitory nerves, formulated by Pavlov fifty years ago, is not tenable in its original form, the inhibitory effect of nerve stimulation on the secretory process cannot be denied altogether. There are indications, for example, that the pancreatic secretion may be inhibited by impulses derived from the central nervous system and transmitted to the pancreas through the vagus nerves. La Barre and Destrée (1928) in cross circulation experiments on dogs established that hypoglycemia produced only in the head of the recipient by insulin or decamethylene-diguanidine inhibited pancreatic secretion in that animal, though the sugar in the blood of its body was at a normal level. Section of both vagus nerves abolished this effect. Dr. Baxter (1932) in my laboratory demonstrated that insulin hypoglycemia has a specific inhibitory effect on the output of enzymes from the spontaneously secreting pancreas of the rabbit. This diminution in the discharge of enzymes by the pancreas was prevented by section of the vagi. Again, La Barre and Soaje-Echague (1938) showed that hyperthermia of the brain centres inhibits pancreatic secretion through the vagus nerves. Harper and Vass (1941), on the other hand, claim that the splanchnic nerves contain secretory-inhibitory fibres for the pancreas. All these conflicting views show that much more evidence is necessary in order to establish beyond doubt the existence of true secretory-inhibitory nerves for the digestive glands. The problem is indeed much more complicated than it appeared to the earlier investigators.

Furthermore, Wedensky (1885, 1892, 1903) established an "optimum" and a "pessimum" of frequency of stimulation for a motor or a secretory nerve. The rhythm of chorda tympani stimulation which produced a maximal secretion was 40 stimulations per second; higher frequencies of stimulation (e.g., 100 to 250 stimulations per second) failed to elicit a secretion. Recently Kupalov and Skipin (1934) found that the optimal rhythm for faradic stimulation of the chorda tympani in the dog was 10 to 30 single shocks per second. In a whole animal the frequency of impulses which originate in the central nervous system and pass along a nerve may undergo changes. As Adrian and Bronk (1928, 1929) showed, the frequency of the impulses in the phrenic nerve of the cat during asphyxiation or in a motor nerve when different strengths of stimulation were applied to the foot, varied from 20 and 30 to 80 and 90 per second respectively. The same might be expected in reflex stimulation of a secretory nerve, and, as we have seen, higher frequencies of nerve impulses do not stimulate but inhibit the secretory activity of a digestive gland.

An interesting experiment was reported by Bülbring and Burn (1942-3), which elucidates some phases of the action of nerve in this case on muscle. The sciatic nerve in a cat was stimulated with single induction shocks at rapid rates. Injection of prostigmine into the common iliac artery increased the tension of the gastrocnemius muscle. When adrenalin was afterwards injected, it lowered the tension of the muscle for a time. If the initial slow rate of nerve stimulation was now resumed, the tension rose again. Prostigmine by itself in large doses caused depression of the muscular tension at fast rates of stimulation. The above-described phenomenon was attributed to excessive preservation of the acetylcholine molecules under the combined effects of prostigmine and adrenalin. The molecules remained attached to the receptors of the cells, preventing the access of fresh molecules of acetylcholine. Perhaps not only adrenalin but other substances or metabolites under certain circumstances might produce a similar effect and depress the activity of a muscle or gland.

Whatever the future solution of the problem of the secretory-inhibitory nerves may be, it can only give us a partial answer to our question concerning the meaning of the double innervation of the digestive glands. This follows from the fact that both stimulation and inhibition of the secretory activity of a gland may be transmitted to it along one and the same nerve. We therefore approached the question of the double innervation of the digestive glands from a different angle, seeking to discover whether the glandular tissue, apart from other structures in a gland, receives nerves from both divisions of the autonomic nervous system. Since most of the digestive glands are composed of several groups of secretory epithelia, it was justifiable to inquire whether each group is innervated doubly or singly, and what the purpose of a double innervation may be, if such exists. But if, for instance, two epithelial groups in a gland each receive their nerve fibres from only one division of the autonomic nervous system, we wanted to know how the stimulation of the sympathetic nerve would affect the action of the parasympathetic, and *vice versa*. Would the two nerves, belonging to different divisions of the autonomic nervous system, act antagonistically, or independently of each other, or even synergistically?

Years of research showed us that in compound glands, like the large salivary glands, for example, each set of secretory epithelia is under the control of a separate nerve—sympathetic or parasympathetic. We did not believe, as had Heidenhain (1868), that each secretory cell is doubly innervated. On the contrary we came to the

conclusion that each cell in a group receives only one nerve fibre from that division of the autonomic nervous system which is in control of this set of epithelial cells. A great amount of evidence was presented in support of this view (Babkin, 1943, 1944).

In spite of the anatomical distinction between the two nerves to the digestive glands, functionally they were dependent to a certain degree on one another. While it was hardly possible to demonstrate a true secretory antagonism between the sympathetic and parasympathetic nerves of the digestive glands, their synergistic effect was clearly evident. This put the digestive glands in a class by themselves, distinguishing them from other internal organs, where the two divisions of the autonomic nervous system usually act antagonistically.

As an example of the synergistic effect of the sympathetic and parasympathetic nerves we shall take first the innervation of the salivary glands, especially that of the submaxillary gland.

SUBMAXILLARY GLAND

The submaxillary gland of the dog or the cat is of rather complicated structure. At least five different types of epithelial cells are to be found in it. At the present moment we are interested in two of these cell groups: the mucous cells, innervated by the parasympathetic chorda tympani, and the serous, or demilune, cells innervated by the sympathetic nerve. The mucous and the serous cells are the chief secretory cells of the submaxillary gland. Other cells are present in the various ducts of the gland, but we have not yet obtained definite proof that these cells possess the property of forming some of the constituents of the saliva, though this possibility is not excluded (cf. Babkin, 1944, pp. 620 ff.).

It was established long ago that faradization of the chorda tympani or of the cervical sympathetic nerve in anaesthetized animals evoked a secretion of saliva. It was only natural to think that under normal conditions impulses from the mouth cavity are transmitted to the submaxillary gland through both nerves. However, a strange fact became evident, namely, that the impulses are transmitted only through the chorda tympani. If, in a normal dog with a permanent salivary fistula, the cervical sympathetic nerve was cut or even the superior cervical sympathetic ganglion extirpated, there was hardly any change in the secretory response of the submaxillary gland to stimulation of the mouth cavity by food or by a rejectable substance, such as a solution of acid. On the other hand, if the chorda tympani was cut and the cervical sympathetic nerve left intact, no impulses from the mouth cavity reached the gland.

This striking discrepancy between the results of faradic stimulation of the two secretory nerves of the submaxillary gland and their participation in the process of reflex salivation impelled us to re-investigate the whole problem of the relationship between the parasympathetic and the sympathetic innervation of the digestive glands.

The early experiments of Dr. V. E. Maevsky in my Odessa laboratory in 1922 dealt with the transmission of impulses from the mouth cavity to the salivary glands through the sympathetic nerve (see Maevsky, 1923). For his experiments Dr. Maevsky had a dog which was equipped with permanent fistulae of the mixed and parotid glands, and in which the chorda tympani had been cut aseptically some time before. Except for a scanty spontaneous "paralytic" secretion, no secretory response was observed or could be obtained from the mixed glands by any stimulation of the mouth cavity with food or other substances. However, if the salivary secretion was stimulated with a small dose of pilocarpine (2.5 mg. per dog), injected subcutaneously, feeding of the animal or injection of an acid solution into its mouth greatly increased the rate of the salivary flow. Analogous results were obtained on anaesthetized dogs. The influence of pilocarpine or the secretory after-effect of chorda tympani faradization was greatly increased by rubbing the mouth cavity of the animal with 0.5 per cent HCl.

In both cases, that is, in a normal animal with permanent salivary fistulae and in an animal under anaesthesia, the reflex from the mouth cavity was undoubtedly transmitted through the sympathetic nerve, for section of the corresponding vagosympathetic nerve in the neck abolished this effect altogether.

The above was a special example of "augmented secretion," a phenomenon which was described long ago by Langley (1889) and which we have studied extensively (see Babkin, 1944, pp. 676 ff.). It might be explained in several ways, as follows:

- (1) In a pilocarpinized submaxillary gland, deprived of its parasympathetic innervation, the increase in the secretion as a result of stimulation of the mouth cavity and the lack of increase after section of the vagosympathetic nerve might be attributed to contraction of the salivary ducts, the myo-epithelial cells of which are under the control of the sympathetic nerve. This explanation is ruled out by the absence in nearly all our experiments of any marked decrease in the secretion during the first few minutes following feeding. Such a decrease would occur if the saliva secreted under pilocarpine in the preceding five minutes' feeding period were merely pressed out of the gland and no additional reflex secretion took place. As a matter of

fact the secretion after the end of feeding was only a little less than before the feeding, but continued to diminish owing to the gradual wearing-off of the pilocarpine effect.

(2) Another explanation might be based on the assumption that during feeding of the animal the tonus of the glandular blood vessels was lowered or a reflex vasodilation might even occur. This supposition is invalidated by the fact that in the dog the sympathetic nerve to the submaxillary gland contains only vasoconstrictor fibres, and also by the fact that after section of the vagosympathetic nerve in the neck there was no increase in the flow of saliva.

However, conditions are somewhat different in the cat, where the sympathetic nerve conveys only a few vasoconstrictor fibres but a great number of vasodilator fibres to the submaxillary gland. Stavraky (1942) demonstrated that in the cat the secretion from a pilocarpinized submaxillary gland, after the chorda tympani had been cut, was greatly increased by intravenous administration of very small doses of adrenalin. Such doses of adrenalin were unable to stimulate the salivary secretion by themselves when acting on a non-pilocarpinized gland. Stavraky's explanation of this phenomenon of increased secretion was that it was due to vasodilation but also to increased permeability of the secretory cells produced by the adrenalin. Nevertheless it could not be denied that there was also a true co-operation of the sympathetic and parasympathetic secretory stimulants in these experiments.

Whatever might have been the mechanism of the increased secretion observed in all these experiments, it is evident that there was synergistic action of the two stimulations. In Maevsky's experiments in all probability a summation of two secretory effects—parasympathetic and sympathetic—was involved. In Stavraky's experiments there was apparently co-operation between the sympathomimetic and parasympathomimetic substances: pilocarpine stimulated the secretory cells and kept the glandular blood vessels moderately dilated and adrenalin acted chiefly by further augmenting the blood supply to the gland and increasing the permeability of the secretory cells.

In order to avoid the use of drugs and deal only with natural stimulants of salivary secretion we turned our attention to the submaxillary gland where the chorda tympani had been previously cut, resulting in the so-called "paralytic" secretion. Our aim was to find out what influence would be exerted on such a gland by sympathetic and parasympathetic stimulation. The first experiments were performed by Maevsky in the year 1922 in my Odessa laboratory and reported by me at the International Physiological Congress in Edin-

burgh in 1923 (Babkin, 1924). These experiments were repeated in my McGill laboratory by F. C. MacIntosh, A. J. Fleming, and H. E. Rawlinson from 1935 to 1937. I mention these facts because our conception of the innervation of the salivary glands had changed fundamentally between 1923 and 1935. In the early period of our work we based our experiments on the assumption, then upheld by practically all physiologists, that every secretory cell may be doubly innervated, receiving nerve fibres from both the sympathetic and the parasympathetic nervous system. When we administered to an animal a drug such as pilocarpine or evoked the paralytic secretion of the submaxillary gland, we referred to the "increased excitability" of the secretory cells as a whole without discriminating which cells were affected in each case. Our later investigations convinced us that the different groups of secretory cells in a gland are innervated separately—for example, in the submaxillary gland of the cat the mucous cells receive nerve fibres from the parasympathetic nerve and the serous cells nerve fibres from the sympathetic. This new conception of the innervation of the secretory cells made it imperative for us to repeat and extend our early experiments.

When the chorda tympani has been cut aseptically, the submaxillary gland produces a very scanty "paralytic" secretion of thin saliva (e.g., one drop every twenty minutes, in a dog). It seemed natural to think that in the "paralytic" gland it is the cells innervated by the parasympathetic nerve that are affected. However, the histological investigations of Langley (1885), of Maximow (1901), and of Rawlinson (1935) in our laboratory showed that very little change took place in the mucous cells. Although these cells became smaller, they had quite a normal appearance and did not show any morphological signs of secretory activity. The serous (demilune) cells, on the other hand, underwent marked change during the whole period of the "paralytic" secretion, which ordinarily lasts from five to six weeks (observations were continued for forty-three days in Rawlinson's experiments). These changes undoubtedly testified to the secretory activity of the cells.

In all our experiments the "paralytic" gland showed a far greater response to sympathetic nerve stimulation or to adrenalin than the normal submaxillary gland in the same animal. The following facts were established by Maevisky on the cat (Table I):

(1) The threshold of stimulation of the sympathetic nerve was much lower for the "paralytic" than for the normal submaxillary gland.

(2) The secretion evoked from the "paralytic" gland by a small dose of adrenalin administered intravenously was three to four times greater than that from the normal gland, and lasted about twice as long.

TABLE I

"Paralytic" secretion of the submaxillary gland in the cat. The chorda tympani on the left side was cut aseptically several days before the acute experiment (column 2), that on the right side at the beginning of the experiment. The cervical sympathetic nerves on both sides were also cut at the beginning of the experiment. Saliva was measured in divisions of a graduated tube. Experiments A and D were on the same animal.

1 Experiment	Left (operated) side				Right (normal) side		
	2 Days after section of chorda tympani	3 Coil cm.	4 Volume of secretion divisions	5 Total time of secretion	6 Coil cm.	7 Volume of secretion divisions	8 Total time of secretion
A. Threshold of stimu- lation	12	28	9¼	1'15"	23	1	30
"	..	30	4¾	45"	24	¾	45"
"	..	32	2½	1'00"	26	0
B. 1 cc. of 1:1,000 adrenalin	10	..	31	3'30"	..	9	1'30"
"	35¾	3'00"	..	8½	1'30"
C. Duration of after- effect	35	20	49¼	6'00"	19	10	45"
D. Two minutes' dispnoea	12	..	24	3'00"	..	0

(3) The secretory after-effect of fifteen seconds' stimulation of the sympathetic nerve lasted six times as long as that of the normal gland and produced five times as much saliva.

(4) Two minutes' dispnoea produced a secretion from the "paralytic" gland but none from the normal gland. This fact had been already observed by Langley (1885). Since, as is now known, as-

phyxiation of an animal causes a discharge of adrenalin from the suprarenal glands, this form of experiment actually involved a sympathomimetic effect.

The facts established by Maevsky were confirmed in the Physiological Laboratory at McGill University by Fleming and MacIntosh (1935) and MacIntosh and Rawlinson (1935). In this work two factors were taken into consideration which were not known in the twenties at the time of Maevsky's investigations: (1) the separate innervations of the mucous and demilune cells of the submaxillary gland and (2) the chemical transmission of nerve impulses. In all the experiments then performed by these investigators on cats special attention was paid to the circulation in the submaxillary gland, the aim being to evaluate and whenever possible to exclude its influence on the secretory process.

Fleming and MacIntosh established the fact that a small dose of adrenalin not only produces a greater salivary secretion from a "paralytic" than from a normal gland but also causes greater vasodilatation in the former. The maximum of the secretion did not coincide with the maximal vasodilatation and the secretion even began to decline while the increase in the flow of blood through the glandular blood vessels was at its height. Therefore, the greater response of the "paralytic" gland than of the normal gland to adrenalin could not be attributed to the more pronounced vascular effect produced by this substance in the paralytic gland. It was due chiefly to a greater response from the secretory cells themselves. However, the fact of the greater vasodilatation in the paralytic gland under the influence of adrenalin was of some importance, for it showed that the blood vessels as well as the secretory cells become more sensitive to adrenalin after section of the parasympathetic nerve.

To obtain additional proof of the synergism of the sympathetic and parasympathetic effects on the submaxillary gland another type of experiment was performed by MacIntosh and Rawlinson (1935). In a normal cat they paralysed the chorda tympani with a small dose of atropine. No secretion of saliva could then be obtained by faradization of the chorda tympani. The effect of stimulation of the sympathetic nerve or of intravenous administration of a small dose of adrenalin was tested before and after an ineffective faradization of the chorda tympani. In order to avoid the effect of the vasodilatation which is produced by stimulation of a lightly atropinized chorda tympani they did not apply the sympathetic or sympathomimetic stimulation to the gland until thirty to sixty seconds after the end of faradization of the parasympathetic nerve, when the vaso-

dilatation was past. In some experiments both the secretory and the vasodilator fibres of the chorda tympani were completely paralysed by atropine. These experiments gave the same results as the previous ones.

In all instances the preliminary faradization of the paralysed chorda tympani definitely increased the effect of sympathetic or sympathomimetic stimulation of the submaxillary gland. In addition, these experiments confirmed an old observation of Langley (1901-2), namely, that the administration of adrenalin can temporarily restore the secretory effect of a chorda tympani paralysed by atropine.

There is no doubt therefore that in the salivary glands the sympathetic and parasympathetic nerves are able to act synergistically under certain circumstances.

What interpretation can be given to all these facts? The real cause of the paralytic secretion is still obscure. Of the various theories put forward, Langley's (1885) explanation of this peculiar phenomenon seemed to be the most feasible and we adopted it with certain modifications. It may be reasonably supposed that a continuous weak activity of the postganglionic neurons of the severed chorda tympani is the cause of the paralytic secretion. The impulses of the nerves are not strong enough to evoke the secretory activity of the mucous cells which they innervate but are capable of producing a continuous small discharge of the parasympathetic chemical transmitter. The latter, presumably acetylcholine, made more susceptible the serous cells innervated by the sympathetic nerve and the muscles of the glandular blood vessels, to sympathetic and sympathomimetic stimulation. Since the sympathetic innervation was intact, the central nervous system could conceivably send impulses to these structures. Thus in the experiments on the paralytic submaxillary gland we had a demonstration of Langley's "augmented secretion" in another form, when the response of the secretory cells to stimulation of the sympathetic nerve was increased by stimulation of the parasympathetic nerve. Whether the "paralytic" gland was more sensitive to parasympathomimetic stimulation than the normal gland is not clear. The data concerning the comparative effect of these drugs on the two glands in one and the same animal are controversial (cf. Babkin, 1944, p. 668).

In the experiments on the atropinized submaxillary gland a seemingly ineffective stimulation of the chorda tympani, as Gibbs and Szelöczy (1932) had observed, liberated acetylcholine. It is very probable that in our experiments on an atropinized submaxillary gland, acetylcholine, which was liberated by stimulation of the chorda tympani, increased the response of the demilune cells to sympathetic

stimulation. What parts of the neuroglandular apparatus of these cells were affected by the parasympathetic chemical transmitter is uncertain.

Another important point was brought out in our experiments on the submaxillary gland. The facts demonstrated could not be attributed to direct electrical transmission of impulses from the nerve to the secretory cell. It seemed more logical that a chemical transmitter of the nature of acetylcholine spreads from the mucous cells, innervated by the parasympathetic nerve, to the serous cells and increases the responsiveness of the latter to sympathetic stimulation or adrenalin. No such phenomenon was ever observed in the parotid gland, which possesses only one type of secretory cells and in which no paralytic secretion could be observed after section of its parasympathetic nerve.

It is of significance that the percentage of acetylcholine equivalent in a paralytic submaxillary gland was only about half that in a normal gland (Chang and Gaddum, 1933). This was not due to an increase in the cholinesterase content of a gland deprived of its parasympathetic stimulation (MacIntosh, 1937) but probably to a continuous, slow use of its chemical transmitter.

To summarize the results obtained on the large salivary glands, the investigation of which in the past contributed so much to the knowledge of the secretory process in general, we have reached the following conclusions.

There are two ways in which the co-operation of the sympathetic and parasympathetic nerves of the salivary glands is achieved.

(1) There is a true synergism of the sympathetic and parasympathetic impulses acting on the secretory cells, as our experiments definitely established. The parasympathetic chemical transmitter not only stimulates those secretory cells which are innervated by the parasympathetic nerve, but also increases the excitability of the secretory epithelium, whose activity is controlled by the sympathetic nerve, and *vice versa*.

(2) Regarded as one whole, the salivary gland with its various components—secretory epithelium, blood vessels, excretory ducts, and so on—displays both synergistic and antagonistic activity. The co-operation of the secretory and vasodilator nerves ensures a copious production of saliva, and the contraction of the excretory ducts, which are under the control of the sympathetic nerve, aids its evacuation from the gland. Though the salivary glands are not supplied with true secretory-inhibitory nerves, strong stimulation of the sympatho-adrenal system, as, for example, by fear, arrests the production of

saliva by means of powerful vasoconstriction. In fright the mouth becomes dry. However, it is not only in such extreme cases that the vasomotor nerves play an important role; presumably they do so also in cases of normal reflex salivary secretion. The narrowing of the glandular blood vessels at the end of the secretory period helps to diminish the formation of unnecessary saliva by cutting the supply of blood to the secretory cells.

GASTRIC GLANDS

In the gastric glands the relationship between the sympathetic and the parasympathetic innervation is far more complicated than in the salivary glands and is not well understood. The muscular sheath of the stomach of course possesses a double, antagonistic innervation—motor and inhibitory, parasympathetic and sympathetic. But there is no clear proof that the sympathetic nerve supplies the gastric glands with secretory fibres which might stimulate the production of acid gastric juice. For example, experiments performed by S. Baxter in our laboratory on dogs and cats showed that prolonged faradization of the splanchnic nerves or administration of adrenalin evoked a secretion not of acid gastric juice but of alkaline mucus (see Babkin, 1944, pp. 241 ff.).

In spite of these negative results it cannot be stated categorically that the impulses conveyed along the splanchnic nerves or the administration of adrenalin exert no effect whatever on gastric secretion. It is true that in most instances an inhibition of gastric secretion was observed when sympathetic or sympathomimetic stimulation was applied to the stomach. This might be explained as the result of strong constriction of the gastric blood vessels (cf. Babkin, 1928, pp. 324 ff., and 1944, pp. 241 ff.). However, several investigators observed a very scanty gastric secretion on stimulation of the splanchnic nerves or administration of adrenalin. It was interesting to note that these effects could be seen to best advantage in a whole stomach or a stomach pouch deprived of vagal or sympathetic innervation. Thus, Baxter in our laboratory cut both splanchnic nerves aseptically in cats forty-eight to ninety-six hours before an experiment. As a result, there appeared a spontaneous slow secretion of alkaline gastric mucus of very low peptic power (which might be called a "paralytic" secretion). Stimulation of the partly degenerated splanchnic nerves increased it. If a massive dose of adrenalin was then injected, the spontaneous mucous secretion was inhibited for one or two hours, but thereafter it increased in volume and became slightly acid and very rich in pepsin (see Babkin, 1944, pp. 246 ff.).

A probable explanation of these facts might be that adrenalin increased the permeability of the peptic and parietal cells to a parasympathetic chemical transmitter which was liberated in very small amounts by the postganglionic vagal fibres. Under normal conditions it is not present in sufficiently strong concentration to stimulate those cells in the gastric glands which secrete acid and pepsin. The striking effect of adrenalin and in a lesser degree of stimulation of the sympathetic nerve, in increasing the permeability of the secretory cells was demonstrated in our laboratory in the case of the submaxillary gland (Hebb and Stavraky, 1936; Langstroth, McRae, and Stavraky, 1938; Komarov and Stavraky, 1940).

Thus many experiments involving sympathetic and sympathomimetic stimulation of the gastric mucous membrane showed that a certain kind of synergism, perhaps indirect, nevertheless exists between the sympathetic and the parasympathetic nerves to the peptic glands.

It was evident that this problem could not be abandoned at that stage. In our laboratory D. A. Ross and L. I. M. Coleman began to

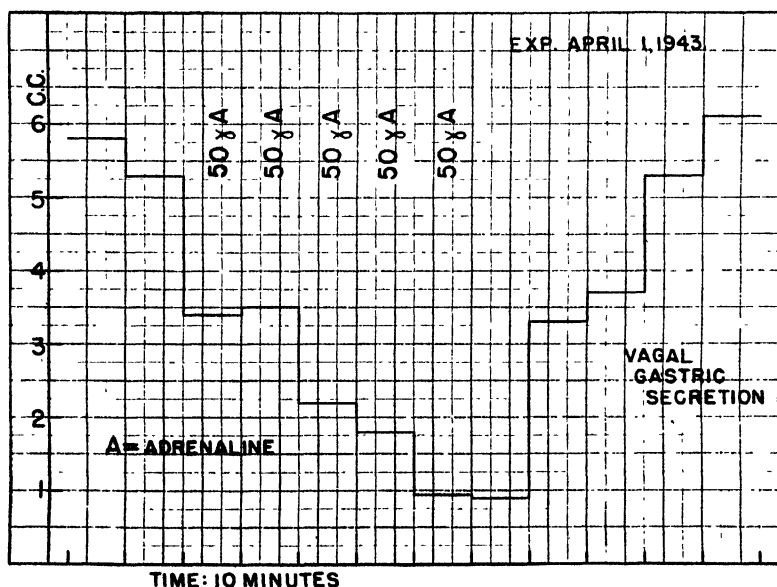


FIGURE 1.—Inhibition of the vagal gastric secretion by adrenalin. Experiment of April 1, 1943. Dog, 7.4 kg., anaesthetized with chloralose and urethane. Gastric fistula made; oesophagus and pylorus tied. Left vagus nerve stimulated with Ross's electrode. Adrenalin injected intravenously.

reinvestigate the relationship of the sympathetic and parasympathetic nervous systems in respect of the gastric glands. Unfortunately, owing to the war, this work remained unfinished. Nevertheless some preliminary experiments indicated that the effect of small doses of adrenalin, superimposed on parasympathetic stimulation of the gastric glands, is not uniform and is not always inhibitory. It is true that in the dog in most cases the gastric secretion obtained by faradization of the vagus nerves, is markedly diminished even by such small doses of adrenalin as 50 micrograms, injected repeatedly (Fig. 1). This inhibition was not due to general vasoconstriction, for after each rise of blood pressure produced by intravenous injection of 50 micrograms

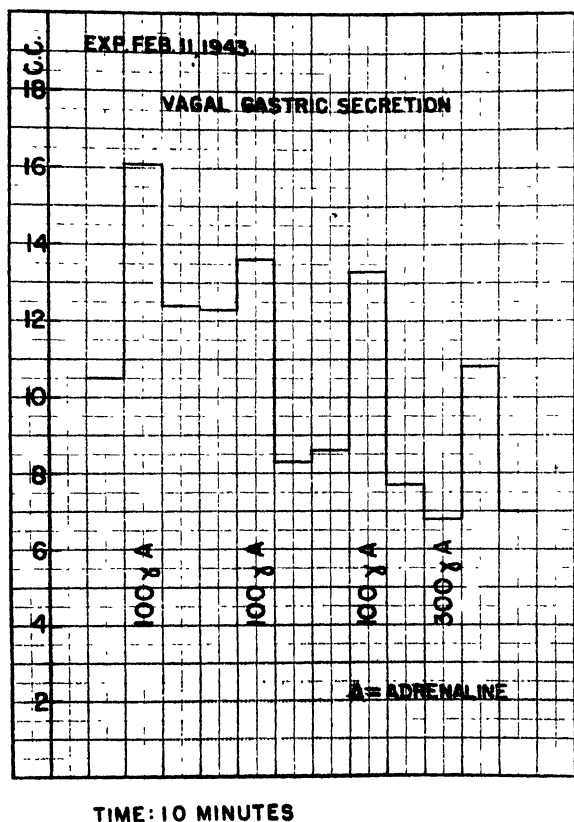


FIGURE 2.—Synergistic secretory effect of vagal stimulation and adrenalin. Experiment of February 11, 1943. Dog, 7.0 kg., anaesthetized with chloralose and urethane. Gastric fistula made; oesophagus and pylorus tied. Left vagus nerve stimulated with Ross's electrode. Adrenalin injected intravenously.

of adrenalin it fell again to practically its previous level. Of course, one cannot exclude the possibility that adrenalin produced a local constriction of the blood vessels of the stomach and thus diminished the production of gastric juice.

However, in some instances even somewhat larger doses of adrenalin (e.g. 100 micrograms) increased the vagal secretion of gastric juice (Fig. 2). No analysis of this phenomenon could be performed and we cannot say whether this positive effect of adrenalin was due to an increased blood supply to the gastric glands or an increased permeability of the secretory cells to the parasympathetic chemical transmitter. Nevertheless it is evident that the action of adrenalin on the gastric secretory mechanism may be either antagonistic or synergistic to the vagal impulses.

PANCREATIC GLAND

We found that quite special conditions existed in the pancreatic gland. Like the salivary glands the pancreas has a double innervation—parasympathetic (vagal) and sympathetic (splanchnic). However, it was demonstrated in our laboratory (Babkin, Hebb, and Sergeyeva, 1939) that both the vagus and the splanchnic nerves convey cholinergic impulses to the secretory cells of the pancreas. There cannot therefore be any actual antagonism between the secretory nerves of the pancreatic gland. Nevertheless administration of adrenalin readily inhibits pancreatic secretion. This is to be attributed, as Edmunds (1909-10, 1910-11) and Mann and McLachlin (1917-18) showed, to the excessive sensitivity of the pancreatic blood vessels to adrenalin. A plethysmogram of the pancreas indicated that a considerable time after the injection of adrenalin the blood vessels of the gland were still contracted, although the general blood pressure had long since returned to the normal level.

THE RELATION OF ACETYLCHOLINE AND ADRENALIN IN THE NERVOUS SYSTEM

During the past few years Burn and Bülbirg (see Burn, 1945) have studied the effect of adrenalin on the transmission of impulses to different nervous structures or on the action of acetylcholine on such structures, namely, the spinal cord, motor nerve, neuromuscular junction, and sympathetic ganglion. They came to the conclusion that low concentrations of adrenalin augment the effect of acetylcholine, while higher concentrations of adrenalin depress it. These findings are in agreement with our own results, for all our experiments

involved stimulation of the glandular tissue through its nerves or by means of sympathomimetic or parasympathomimetic drugs. Both in our experiments and in those of Burn and Bülbirg the actual cause of potentiation of one chemical transmitter or its substitute by another remained obscure.

ELASMOBRANCH FISHES

Why do not the digestive glands possess an antagonistic autonomic innervation as do other organs? One reason might be that the digestive glands are composed of different sets of secretory epithelia and that each of these possesses its own sympathetic or parasympathetic innervation which stimulates it to activity. This theory is well adapted to explain the phenomena that occur in the submaxillary gland of the dog or cat. The history of the development of the salivary glands is related to that of the development of the branchial arches, where ectoderm meets endoderm, and it is possible that the different sets of secretory epithelia are derived from these two embryological layers.

There is another fact which should be taken into consideration when the functions of the alimentary canal are discussed, namely, that it is less specialized in character than other organs. The alimentary canal has retained many features of its primitive arrangement, which are revealed in its multiple functions—secretion, motility, absorption, and formation of numerous hormones. During embryonic life the autonomic nerves are added to the alimentary tube when it is already formed. Was the alimentary canal supplied with excitatory and inhibitory nerves from the very beginning, or did the autonomic nervous system undergo evolution, during the course of which the functional differentiation of the nerves took place? This problem greatly interested me and my associates. We did not study the functional development of the autonomic nervous system in embryos but approached the problem from a different angle. Every summer for a number of years we worked at the Atlantic Biological Station, St. Andrews, New Brunswick, using as material for our investigations a very primitive form of vertebrate—the Elasmobranch fishes (chiefly the skates, *Raja diaphanes*, *Raja erinacea* and *Raja stabuliforis*). It seems that in the course of the evolution the stomach as well as, presumably, the rest of the gastro-intestinal tract, developed gradually within the vertebrates. Therefore the regulation of the gastro-intestinal activity in fishes, and especially in Elasmobranchs, is characteristic of a rather early stage in the evolution of this function. (Cf. Barrington, 1942).

The cartilaginous fishes represent a very ancient group. Their evolution began in the early Silurian era or perhaps even sooner, that is, approximately 400 million years ago (Romer, 1933). These animals have a completely developed gastro-intestinal tract. They possess an oesophagus, a well-formed stomach, structurally similar to the stomach of the higher vertebrates, and a duodenum, spiral intestine, colon, and rectum. The liver and the gall-bladder and pancreas discharge their secretions through their respective ducts into the duodenum. The gastro-intestinal tract of the skate receives its nerve supply from the vagus nerves and from the sympathetic nerves derived from the ganglia which lie on either side of the vertebral column. We were much interested in discovering how the functions of this morphologically well-developed gastro-intestinal tract were regulated and what relations existed between the two divisions of the autonomic nervous system which innervated the same structures in the tract. The chief facts which we obtained, and which in part confirmed the results of other investigators, may be summarized as follows (the voluminous literature on the subject is cited in the papers mentioned below):

The main conclusion to which we came was that in such ancient animal forms as the Elasmobranch fishes the nerves of the autonomic nervous system had never become differentiated into excitatory and inhibitory. Much later, during the course of evolution, these nerves acquired in other species antagonistic functions. The same conclusions were reached independently of us by Young (1933), who believes that "the sympathetic and parasympathetic systems of mammals, with their complex balance of excitation and inhibition, are a recent development."

Our point of view is supported by the following facts:

(1) In the skate the muscles of the stomach are supplied with both vagal and sympathetic innervation; nevertheless the effects produced on gastric motility by stimulation of the vagus and the sympathetic nerves respectively are not antagonistic but synergistic, both nerves being motor. (Babkin, Friedman, and MacKay-Sawyer, 1934).

(2) Isolated strips were excised from various parts of the stomach of the skate. A study of their reaction to different drugs showed that the motility of all parts of the viscus (with one exception) is increased by acetylcholine, pilocarpine, and adrenalin. Adrenalin in a concentration of 1:250,000 (in a saline bath) stimulated all parts of the stomach, except the antral region near the pyloric canal. The antral region was so hypersensitive to adrenalin that even with a concen-

tration of 1:1,000,000 of adrenalin its movements were inhibited. However, if smaller concentrations of adrenalin were used (e.g., 1:2,000,000) or the excitability of a strip of antral tissue was lowered by being kept for three or four days in saline at 0° C., contractions could be noted (Nicholls, 1933a). Thus we could observe the first manifestations of an inhibitory effect of a sympathomimetic substance. It seems that during the evolutionary history of the vertebrates the inhibitory properties of the sympathetic nervous system in relation to the muscles of the alimentary canal developed very gradually. For example, in amphibians the sympathetic nerve to the stomach still retains its motor functions.

(3) Acetylcholine stimulates all parts of the intestine (spiral intestine, colon, and rectum). The same effect was produced by adrenalin as a rule. (In two experiments, but in no others, the motility of the spiral intestine was inhibited for some unexplained reason (Nicholls, 1933b)).

(4) The heart of the Elasmobranch fishes is supplied by the vagus nerve, stimulation of which produces inhibition of the contractions. This fact was known long ago (for literature, see Huntsman, 1931, and Young, 1933). The existence of a sympathetic innervation of the heart is doubtful and the presence of sympathetic fibres to the heart is very much disputed. However, E. Huntsman observed that adrenalin caused both augmentation and acceleration of the contractions of the isolated skate heart. Acetylcholine arrested the contractions of the heart. The effects of adrenalin and acetylcholine were abolished by atropine. The heart tissue of the Elasmobranchs therefore is responsive to sympathetic stimulation, although the heart is not connected with the sympathetic ganglia. Since the Elasmobranchs possess chromaffin tissue in the form of chromaffin bodies lying close to the sympathetic ganglia, it may be supposed that under certain circumstances the heart of these animals is stimulated by adrenalin through the blood.

(5) Adrenalin was shown to produce a very striking pressor reaction in the skate, and acetylcholine likewise, when these drugs were injected intravenously in the whole animal (MacKay, 1931). When the large abdominal arteries of the skate were isolated, the circular and longitudinal layers of muscle contracted in response to adrenalin and acetylcholine. This may explain in part the pressor effect of these substances when administered intravenously to the whole animal (Babkin, Bowie, and Nicholls, 1933).

(6) Histamine, administered intravenously to the skate even in very large doses, had neither a depressor nor a pressor effect on the

circulation. Although histamine does not produce vasodilatation in the frog, extracts of frog's skin revealed the presence of histamine-like and choline-like substances, the former producing a histamine-like effect in mammals. It was of interest to determine what substances could be extracted from the skate's skin. The following results were obtained. The histidine-arginine fraction of the extracts produced a slight rise of blood pressure in the skate and a histamine-like reaction in human skin, the lysine fraction a marked rise of blood pressure and inhibition of the heart rate in the skate (MacKay-Sawyer and Komarov, 1933).

(7) M. E. Sawyer (1933), independently of us, studied the reaction produced by adrenalin and acetylcholine on a sheath of muscle lying in the mesentery between the oesophagus and the large intestine in the skate and the dogfish, and found that both these substances caused it to contract.

(8) With respect to the digestive glands, it seems that in the skate these glands had never been supplied with any fibres from the autonomic nervous system. The existence of a secretory innervation of the stomach (Babkin, Chaisson, and Friedman, 1934) and of the pancreas (Babkin, 1929, 1931) in the skate is very doubtful. The mechanism responsible for the secretion of gastric juice is humoral or vascular. Histamine has no effect on the gastric glands. The secretion of pancreatic juice is stimulated presumably by secretin formed under the influence of acid in the intestinal walls.

All these facts support the view, expressed above, that in Elasmobranch fishes, which represent a rather early stage in the development of vertebrates, no definite differentiation of the autonomic nerves into excitatory and inhibitory had been achieved. These nerves are predominantly excitatory and only the vagus branches, which innervate the heart, exercise a strong inhibitory function. However, the heart is without any sympathetic innervation. To this may be added that Bernheim (1934) has seen that an intestinal strip from teleost fishes of the genus *Epinephelus* in a state of contraction under the influence of acetylcholine was contracted further by the addition of adrenaline. The effect of acetylcholine on the musculature of the stomach of the amphibian *Necturus maculatus* or mud puppy, as was shown by Friedman (1935) in our laboratory, was antagonised when adrenaline was used in relatively high concentrations, and enhanced when employed in a greater dilution.

It is noteworthy that even in the mammals there is no sharp division of the autonomic nerves into excitatory and inhibitory as far as certain functions of the gastro-intestinal tract are concerned. For

example, McSwiney (see 1931) and his co-workers demonstrated in respect of the stomach of the dog or cat that the excitatory or inhibitory motor effect of the vagus or the sympathetic nerve depends on whether the muscles of the viscus are in a state of contraction or relaxation at the moment of nerve stimulation. The stomach muscles of the foetal cat usually reacted to adrenaline, which followed acetylcholine, antagonistically, but in some cases a synergistic effect was observed depending on the concentration of adrenaline (Friedman, 1936).

CONCLUSION

From this review of the functional relationship of the sympathetic and parasympathetic innervations of the digestive glands it is evident that the two divisions of the autonomic nervous system co-operate, enhancing each other's secretory effect. It is much easier to observe the synergism of the sympathetic and parasympathetic impulses to the glandular tissue than their mutual antagonism. That the latter is a reality cannot be denied, but more evidence is required in order to prove it beyond doubt.

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EFFECT OF HEAT ON THE LIGHT BEHAVIOUR OF FISH

By C. W. ANDREWS

Presented by A. G. HUNTSMAN, F.R.S.C.

INTRODUCTION

HEAT and light of varying intensities are factors in the environment of fish. In the Petitcodiac River system, New Brunswick, it is not uncommon during warm summer weather for the temperature to rise to such a peak as to approach the lethal for some species of fish. During the summer of 1942 many young salmon died in the Petitcodiac system from this cause.

It was observed in the Moser River, Nova Scotia, in 1939 (Huntsman, 1942) that salmon at a very high temperature became insensitive to light and reversed their usual vertical distribution, the largest individuals being most affected and coming nearest the surface rather than going farthest down.

Experiments have been performed by the writer to study the light behaviour of the common sucker (*Catostomus commersonnii*) at various temperatures, chiefly temperatures approaching the lethal point.

METHODS

In general the experimental method and technique were similar for both field and laboratory experiments. The experiments were performed in a galvanized tank measuring 4 ft. \times 2 ft. \times $\frac{1}{2}$ ft. A deeper wooden tank measuring 4 ft. \times 2 ft. \times 2 ft. was used when a depth of more than 6 inches was required. The tank was divided into two compartments of similar size by a board partition. A space extended across the bottom so that the fish could pass freely from one compartment to the other. A black curtain was placed over the tank to shut out the light. The curtain was fastened at the middle to the top of the partition and at each end of the curtain a roller was attached. Thus the compartments could be exposed as required.

When an experiment was to be performed in the field the fish were collected from the river by means of a hand seine and as soon as possible they were placed in the experimental tank. In the laboratory the fish were kept in storage tanks. In both places the fish remained in the experimental tank at least thirty minutes before the experiment began.

The fish were stimulated by exposing first one and then the other compartment to light. The movement of the fish from one com-

partment to the other was recorded. Five alternate exposures were given for each temperature tested. For the field experiments the temperature was increased (1) by means of the sun's rays or (2) by controlled heating at a rate of 1 degree increase per 5 minutes. In the laboratory (2) was used in all cases. Temperatures were recorded in degrees Centigrade in all experiments.

EXPERIMENTAL

Effect of depth on light behaviour

In nature the common sucker is found in muddy pools and quiet waters. In pools about 12 inches in depth they arrange themselves in the following order in the day-time: underyearlings 1 to 6 inches below the surface, yearlings and two-year-olds at the bottom. Larger fish are found in deeper waters; i.e., the underyearlings are found in the region of highest light intensity and larger fish are found in successively lower light intensities, since light is absorbed by the water and decreases in intensity with an increase in depth of the water.

Experiments on two-year-old suckers showed that in water 8 and 12 inches deep they were negatively phototactic, i.e., they swam into the dark compartment when exposed to light. At 14 inches they remained in either compartment indifferently. At 16 inches and in deeper water they became positively phototactic, i.e., they swam into the light compartment, the reverse response of what was observed for a depth of 12 inches. On decreasing the depth again to 8 inches the fish reverted to their negatively phototactic behaviour. This suggests that the fish preferred a certain light intensity which was found at a depth of between 12 and 16 inches.

Behaviour of underyearlings and yearlings at temperatures short of the lethal

Underyearlings were positively phototactic. This corresponds with their behaviour in nature since they are found near the surface where the light intensity is highest. This type of phototaxis continued up to and including 30.5°. At 31° there was an indication of failure to respond to the light stimulus. At 31.2° the failure was still more marked and when 50 per cent of the fish failed to respond they were said to be insensitive to light. At the insensitivity temperature the fish distributed themselves at random over the bottom of the tank. Insensitivity continued until the lethal temperature was reached, which for this rate of increase (1 degree per 5 minutes) was 33.2°.

That no permanent change had taken place was shown by the

fact that underyearlings, even after having been exposed to the temperature of insensitivity ($31.6-32.7^{\circ}$) for 65 minutes, again became sensitive to light at 31.2° when the temperature was gradually decreased. Also yearlings which were negatively phototactic, i.e., swam into the dark compartment, after having been exposed to the temperature of insensitivity ($32.7-33.0^{\circ}$) for 2 hours, were again sensitive to light when tested at 26.1° .

Effect of age on the temperature of insensitivity to light

Experiments were performed at the Pollett River, New Brunswick, August 2-5, 1944, on underyearlings, yearlings, and two-year-olds to determine the effect of age on the temperature of insensitivity. Age was estimated from the length of the fish. A total of 58 fish was used. Underyearlings (1-3 cm. in length) became insensitive at $33.5-34.2^{\circ}$, yearlings (6-9 cm. in length) at $33.6-33.9^{\circ}$ and two-year-olds (10-14 cm. in length) at $28.5-29.2^{\circ}$. As the age increased the temperature of insensitivity decreased.

The effect of acclimation temperature on the temperature of insensitivity to light

This experiment was performed under laboratory conditions. Tests were made on each of four groups of fish, which had been acclimated to 5° , 12° , 17° , and 22° respectively. Those acclimated to 5° had been living at that temperature for one month. At least one day per degree of increase was allowed for each of the higher acclimation temperatures. Figure 1 shows that as the acclimation temperatures increased the temperature of insensitivity also increased. For example: at an acclimation temperature of 5° the temperature of insensitivity to light of a certain intensity was 20.3° and at an acclimation temperature of 22° the temperature of insensitivity to light of the same intensity was 32.5° . At a light intensity 100 times less, insensitivity set in at a lower temperature; for example, when the acclimation temperature was 5° , the temperature of insensitivity was 17.8° and when the acclimation temperature was 22° the temperature of insensitivity was 28.5° . Each point on the curve represents at least ten fish.

Effect of increase in light intensity on the temperature of insensitivity

In this experiment four light intensities were used. The relative intensities of these lights were 1, 10, 100, and 1,000. The strongest light used was an electric light of 150 watts suspended three feet above the surface of the water and its approximate strength was 2.5 foot candles as determined by a Weston photometer. A 7.5 watt

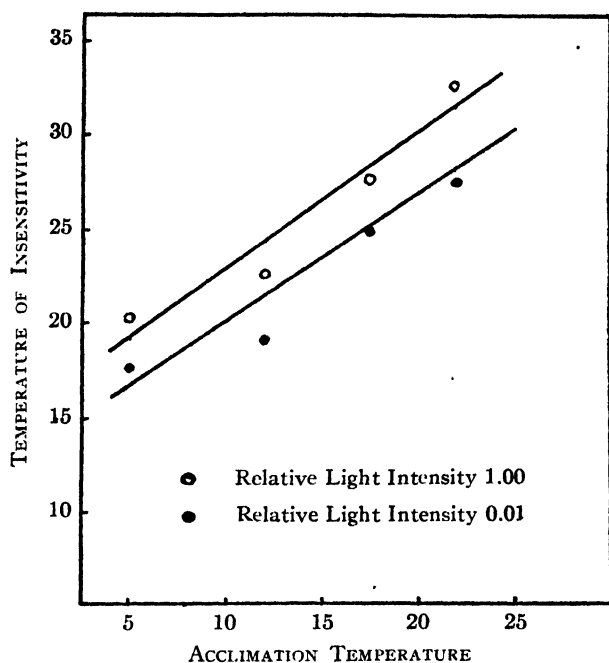


FIGURE 1.—Effect of acclimation temperature on temperature of insensitivity.

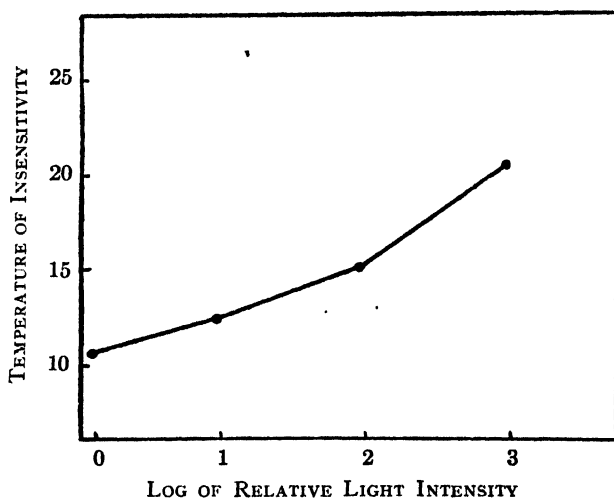


FIGURE 2.—Effect of increase in light intensity on temperature of insensitivity.

light was used for the other strengths, controlled to give various intensities by altering the distance from the surface, and reducing the light emitted by means of an iris diaphragm. The relative intensities were determined by means of a photo cell and galvanometer. The fish were acclimated to 5° C. Figure 2 shows that as the light intensity increased the temperature of insensitivity also increased. For example: when the relative light intensity was 1 the temperature of insensitivity was 10.5° and when the relative light intensity was 1,000 the temperature of insensitivity was 20.5°. Each point on the curve represents five fish.

SUMMARY

The effect of heat on the light behaviour of the common sucker (*Catostomus commersonnii*) has been studied.

Two-year-olds were negatively phototactic in shallow water but they reversed their behaviour and became positively phototactic in deep water.

All sizes tested became insensitive to light at temperatures short of the lethal. If the temperature was decreased below the temperature of insensitivity the fish again became sensitive to light.

As the age of the fish increased the temperature of insensitivity to light decreased.

The temperature of insensitivity varied directly with the temperature of acclimation; i.e., as the acclimation temperature increased the temperature of insensitivity also increased.

The temperature of insensitivity varied directly with the light intensity; i.e., as the light intensity increased the temperature of insensitivity increased.

ACKNOWLEDGMENTS

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FOOD OF FISHES

By C. McLEAN FRASER, F.R.S.C.

WHILE serving as Director of the Pacific Biological Station, Nanaimo, British Columbia, 1912-24, I spent some considerable time in the investigation of the life history of some of the species of fish of commercial importance. Incidental to this, use was made of many an opportunity to examine the stomach contents of freshly caught fish of not only the species especially subject to investigation, but also other species not studied to the same extent, to get a better idea of the nature of the food consumed. The observations were recorded at the time but as there was little interest taken in this phase of the life history then, little use was made of the notes.

The situation is different now since in the research carried on at the Station more emphasis is placed on economics. The food of fishes, consequently, is receiving a full share of attention. Although the observations to which reference has been made were incidental, they were made over several years and at all times of the year. It may, therefore, be an appropriate time to make a study of these observations and present the results as a contribution to the question of the food of fishes as indicated by stomach contents. All the fish were caught in Departure Bay or in the vicinity.

SPRING SALMON

Oncorhynchus tshawytscha (Wallbaum)

Of the spring salmon that were examined, several were noted as "young spring salmon"; these were all taken between March 2 and June 7. They were probably all salmon of the sea-run type that had arrived but recently in the Strait of Georgia from the streams where they were hatched. When they first appear they are usually less than 2 inches in length, but by June 7 they might be as much as 6 or 7 inches. There may be one exception to this as in one specimen, taken on March 4, it is definitely stated that there were yearling herring in the stomach. At that time of the year, a sea-run spring in its first year would scarcely be able to swallow a yearling herring. It is probable that this was a stream-run specimen at the beginning of its second year, since, on the average, a stream-run spring in its second year would be about 4 inches in length at that time. In other cases, the herring used as food were probably all newly hatched, at that period. The scarcity of the stream-run fish in the collection is

not inconsistent with the indication, at that time, that 65 to 70 per cent of the spring salmon in the Strait of Georgia were of the sea-run type.

As in some cases several fish were taken at one time and the nature of the stomach content of the whole lot was noted as one record, the different organisms that were used as food cannot be given as percentages. Herring were mentioned in approximately 30 per cent of the records; euphausiids and amphipods in about 20 per cent; cypris larvae, ostracods, small squid, and ascidian larvae in 7 or 8 per cent. It does not follow that this is the order of preference. At this time of the year the inshore water of Departure Bay and vicinity is "filled" with recently hatched herring. If these are at all desirable as food they could scarcely fail to make up a large proportion of it. Euphausiids and amphipods school up in much the same way but of course the schools are not so large, or at least not so noticeable. The other forms represented in the food may be quite abundant, but they are likely to be more scattered and hence less easily obtained. It may be surmised then that much of what makes up the larger species in the plankton is good food, any of which may be used to give variety as well as nourishment, especially so as there seems to be little variety in the food taken by any one lot of individuals.

There is no indication in these records that the smaller plankton organisms such as copepods, nauplii, zoeae, rotifers, etc., are taken as food.

With the older salmon the time of taking extends over a larger part of the year, but even here there are no records between July 10 and December 4. The records are quite well distributed throughout the rest of the year.

Three specimens cannot have been far away from the "young" salmon, either sea-run, well on in their first year, or stream-run, in the second year. They may serve as a connecting link between the "young" salmon and the older ones. One, caught on July 10, contained young herring; one on December 4, ostracods; one on February 9, annelids and small shrimps—the first two, thus, close to the young salmon food, the third tending towards the older salmon diet.

The remainder form a heterogeneous lot, of any size or age within the limits of the species in the Strait of Georgia. In these, plankton organisms are largely discarded, and with them the most of the invertebrates; fish becomes the chief food ingredient. There is still a residue of annelids and euphausiids in the food of about 10 per cent of the specimens, and in one instance, possibly as a delicacy, the squid (*Loligo opalescens*) appeared. There was some variety in the

fish diet, but herring, i.e., the larger herring, appeared in about two-thirds of the specimens. The nearest approach to the herring are the sticklebacks, in about one-eighth of the specimens. Young spring salmon, young chum salmon, and pilchard appeared even more sparingly.

Here again abundance may be of greater importance than preference. Herring are present somewhere in the Strait of Georgia or in the neighbouring waters throughout the year. It is true that they are seldom seen in Departure Bay during the midsummer months when the surface salinity is low; they move to such channels as Active Pass and the Yucultas, where the water is moving so rapidly that stratification cannot readily take place. Hence there is not a month in the year in which records were obtained where herring did not serve as an article of diet. The other species, with the exception of the pilchard, which must have appeared incidentally or accidentally, do school up in these waters, but only for a rather limited time. One might have expected to find sand launces, since they also school up, but evidently no individuals were caught at the right time to provide any evidence here. Pipefish also appear in large numbers at times but I have never known a spring salmon to use the species as food and do not remember hearing or seeing any record of such.

To give some idea of the capacity of a spring salmon's stomach it might be stated that a salmon weighing 10 lbs., 14 oz., had five mature squid (*Loligo opalescens*) in its stomach; a second, weight not given, had two young spring salmon each about 5 inches long; a third, weighing 18 lbs., had five mature herring; and a fourth, weighing 8 lbs., had twenty-seven undigested sticklebacks.

COHO

Oncorhynchus kisutch (Wallbaum)

The food records for the coho are not nearly so extensive as those for the spring salmon. Coho in this locality are almost all of the stream-run type. When they migrate to sea in the beginning of their second year, as fish 3 to 4 inches in length, apparently they almost invariably disappear from inshore waters. In any case, schools of them such as those of spring or chum salmon, have never been observed in the waters near shore. During the second year they are seldom caught; probably they are too small to be taken with the seine or with the spoon. As they begin their third year in April, or slightly earlier, they become more obviously active as they come near the surface to feed. At this time fishing for them begins (they are then known locally as "bluebacks"), and is continued until they ascend

the streams to spawn in the fall. All of the records were obtained in that relatively short period, viz., April 10 to October 8.

In these cohos in their third year there does seem to be a food preference. While the fry of spring and chum salmon, herring, and ling cod are all so abundant in the spring and early summer, and the more mature stages of herring and capelin in the late summer and fall, and while all of these have been found in coho stomachs, the amount of this type of food is hardly significant when compared with the euphausiid content. The fish gorge themselves on these euphausiids to such an extent that the euphausiids contained may make up 20 per cent of the whole weight of the fish. Apparently it is because of the richness and suitability of this food that the coho is enabled to grow fast enough to double its weight in two months after it shows itself in April. No other invertebrates were recorded.

CHUM SALMON

Oncorhynchus keta (Wallbaum)

There is every indication that some spring and some coho remain in the Strait of Georgia and neighbouring waters throughout all of their marine life. It is different with the chum salmon; they are all sea-run; they make their way down to salt-water while they are still small (approximately 1.5 cm.), some of them before the yolk is all absorbed. They remain in schools for a time, at the most a couple of months, feeding in the inshore waters, after which they migrate to the open sea and are not seen again in the Strait of Georgia until they return to spawn. At this time they are caught with purse seines; they have ceased feeding. In consequence, the only time they can be obtained in the vicinity of Departure Bay to study stomach contents as indicative of the material used as food is in the late spring and early summer of their first year.

The earliest date noted for the first appearance of chum salmon fry in Departure Bay was February 15, and the latest, April 11. All the records of stomach content were obtained during the period from April 27 to June 26.

In the records there is little to show any food preference, so one should be inclined to conclude that almost anything small enough to be swallowed, provided it is large enough to have significant substance, is used as food. If there is any dominance, barnacle larvae, both nauplius and cypris, have it, as these were present in about one-third of the individuals. Diptera and mollusc eggs do not appear so often and yet they were more frequent than any of the others—

copepods, amphipods, crustacean eggs, crustacean sloughs, shrimp and crab larvae, gastropod and pelecypod larvae, and young herring—which appeared more sparsely. There is even greater diversity shown than in the food of spring salmon. Apparently anything that can be swallowed is taken in whether it is digestible or not, although it may be that the crustacean sloughs were mistaken for the organisms themselves. There can be no preference for fish food, since the only young fish obtained, herring, appeared in only one lot of specimens.

HERRING

Clupea pallasii (Cuvier and Valenciennes)

There are much more numerous records of the stomach content of herring than of any other species of fish; possibly there are as many as for all others put together, taken at all times of year and for herring of all ages. It is the only species that I have often watched feeding in the open water. In February, in particular, when the waters of Departure Bay are often smoother and clearer than at any other time of the year, it is an easy matter to stand on the Station float and watch the herring pick up the organisms as they swim by, or to row quietly over the school to see how busy they all are in getting their food supply. It is even possible, in some cases, to follow an individual for some distance as it is taking its meal. On one occasion, for instance, I walked along the float for about eighteen feet, keeping pace with a herring, and in that distance it took in an organism (possibly more than one) every foot of the distance, on an average. I have never estimated the number of copepods that a mature herring could contain, but a young herring (6.6 cm.) approximately six months old, had over three thousand of them in its stomach and median caecum. A mature herring must swim a long way really to fill up. Since one school of herring may and usually does contain many millions of individuals, it would be necessary to use astronomical figures to indicate the number of small organisms that make up a day's food supply for a school.

As most of the records available were used in making the study necessary to prepare the paper on "The Pacific Herring" published in 1922,¹ it is not necessary to go into them at length here. There seems to be little change of diet as the fish grows older, at least after metamorphosis takes place. At all stages copepods make up the principal supply, although at any particular time it may be almost entirely the nauplius or the cypris larvae of barnacles, or it may be

¹Contrib. to Canad. Biol. for 1921, pp. 105-11.

rotifers. When the mature fish come into inshore waters near or at spawning time, the barnacle larvae are very abundant and then they make up the entire stomach content. When the content is entirely of rotifers or copepods, the herring are usually not so close inshore.

Almost every other kind of crustacean larvae found in the vicinity as well as annelid, molluscan, and ascidian larvae, mature amphipods, ostracods, and euphausiids, crustacean and molluscan eggs, and even peridinia, appear on occasion. Herring eggs and young herring fry do not come amiss, so that even the vertebrates do not go unrepresented.

It would be unwise to say whether abundance or preference is of the greater importance as far as this species is concerned.

DOGFISH

Squalus suckleyi (Girard)

There is an abundance of records on the stomach contents of the dogfish but unfortunately nearly all of them were made in association with the study of the herring, and while they do show that at times the herring schools are accompanied by dogfish that rely on them entirely for food, they do not show that other species are not followed and preyed upon, at other times.

With two exceptions, all of the dogfish examined had herring in the stomach if there was any recognizable food material. In the one instance, there were euphausiids, and in the other, a relatively small octopus. Herring of all sizes and ages are used by them. Quite often a school of herring may be discovered when it is near the surface, by observing the dorsal fin of the dogfish cutting the surface as the fish moves about in the school. The dogfish swims along leisurely with the school of herring, and when it becomes so inclined it suddenly increases its tempo for a moment to seize a herring, usually swallowing it whole. When the herring appear in the bay, especially within a month or two of spawning time, they often swim past the float, and then the easiest and quickest way to obtain a few of them for examination is to spear a dogfish. One dogfish may have as many as six mature herring in its stomach. Sometimes, though, the herring is cut in two, the one part only being swallowed. The cut is as definite as if it were made with a sharp knife.

In the water around the cannery wharves it is often possible to see readily the dogfish feeding on the offal. It must be nourishing food for the largest dogfish that I have seen were observed in these locations.

MISCELLANEOUS

Besides these species in which many specimens have been examined there are several others in which the records are very few, usually one to three. In these cases, no general statements can be made, but on the supposition that even a few records give some information and are better than none, they are included here.

Gadus macrocephalus (Tilesius): viviparous perch (*Phanerodon*), pipefish (*Syngnathus*), shrimps.

Lepidopsella bilineata (Ayres): large *Nereis*, shore crabs (*Hemigrapsus*), herring eggs.

Gasterosteus aculeatus (Linn): copepods, barnacle larvae, decapod larvae.

Ophiodon elongatus (Girard): herring, young and mature, hermit crabs and their shells, fish eggs, sixteen species of hydroids in one stomach.

Sebastes pinniger (Gill): herring.

Hexagrammus stelleri (Tilesius): young specimen—small crabs; mature specimens—young *Epigeeichthys*, small crabs, nearly hatched herring eggs.

Scorpaenichthys marmoratus (Ayres): Red sculpin (*Hemilepidotus*), viviparous perch (*Phanerodon*), crabs (*Pugettia producta*, *Cancer productus*, *Hemigrapsus nudus*), shrimps of various species, *Ophiodon* eggs, other fish eggs, vertebrae of unidentified fish. The specimen that contained the red sculpin was a female, 23½ inches long, 16 lbs., with a width of head 7 inches. The sculpin was 12½ inches long, weighed 17 oz., with a width of head 4 inches; it had several shrimps in its stomach still in a good state of preservation.

Hemilepidotus hemilepidotus (Tilesius): bullheads, crabs and shrimps of several species, mussels.

Cymatogaster aggregatus (Gibbons): barnacle larvae. Individuals of this species may be observed throughout the year in the barnacle zone of the piles of the wharf, apparently nibbling at the appendages of the barnacles as they move in and out while the barnacle is feeding.

Taeniotoxa lateralis (Agassiz): herring eggs. They too frequent the barnacle zone around the piles and may feed on barnacles.

RESPIRATORY ENZYME MECHANISMS IN AN INSECT,
WITH REFERENCE TO THE QUALITATIVE AND QUANTI-
TATIVE EFFECTS OF INHIBITORS AS AN APPROACH TO
INSECT TOXICOLOGY*

By KENNETH GRAHAM

Presented by J. J. DE GRYSSE, F.R.S.C.

INTRODUCTION

THE continued need for improved insecticides has necessitated investigations into the site and mode of toxic action of chemicals on insects, and into the relationships between chemical structure and toxicity. The elucidation of the mechanisms by which poisons bring about death in insects establishes fundamental principles by which the introduction of desirable properties into new insecticidal preparations can be achieved in a more systematically directed manner.

A great number of insecticides have been evaluated by dosage-mortality tests (cf. Campbell, 1930; Bliss, 1935; Campbell and Moulton, 1943; Hurst, 1943; and many others), by methods reviewed by Tattersfield (1939), Shepard (1939), and Hurst (1943). Since the value of any insecticide is determined ultimately by its lethal action on the insects against which it is intended to be used, the method provides the practical final criterion for evaluating insecticides. In addition, this method has provided a basis for establishing many of the relationships between chemical structure and toxicity through investigations on families of compounds differing in respect to substituted groups (Tattersfield, 1927; Hoskins, Bloxham, and Van Ess, 1940; Richardson, 1942; Läuger, Martin, and Müller, 1944; and others).

Since killing is an all-or-none phenomenon, it establishes only the general relationship between chemical constitution and toxicity; it does not measure toxicity in relation to specific living functions. Dosage-mortality tests do not generally permit of distinction between non-toxicity *per se* and other limiting factors. On the other hand, a knowledge of the underlying mechanism of toxic action permits the

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classification of poison types on a functional basis and makes possible the selected combination of poisons of different types to give mixtures of enhanced toxicity (cf. Bliss, 1939).

The various known mechanisms by which insecticides kill have been reviewed by Trappmann (1938) and Weed (1943). The range of physiological disturbances set up by poisonous chemicals on insects and other Arthropods includes the following phenomena: nerve lesions (Hartzell, 1943; Wigglesworth, 1941); destruction of fibre tracts and vacuolization of the nerve tissue of the brain (Roy, Ghosh, and Chopra, 1943; Hartzell and Strong, 1944); clumping of chromatin and chromatolysis in nerve cell nuclei (Hartzell and Scudder, 1942); changed action potentials (Roeder, 1939; depression of cardiac activity (Yeager, 1938; Prosser, 1942; Baylor, 1942; Coon, 1942); diminished oxygen consumption (Fink, 1926; Bodine, 1934; Nishikawa, 1938); retarded passage of food through the alimentary canal (Snipes, 1938); degenerative changes in the mid-gut epithelium (Pilat, 1935; Woke, 1940); blood cell degeneration (Tareeva and Nenjukov, 1931; Fisher, 1936; Yeager and Munson, 1942). To this list one might also reasonably consider adding photodynamic action (cf. Tennent, 1942) as a possible influence hitherto not considered in insecticidal action.

With the exception of the work by Bodine, who was, however, concerned with special features of metabolism, particularly in embryonic insects, very little is known of poison action on the chemical systems by which insects derive energy for their life processes. If it be accepted that energy is necessary for function and life (Szent-Györgyi, 1938), then the sustained interruption of energy release in cells must ultimately arrest organ function and result in death. A clearer knowledge of these processes in insects and an understanding of the mechanism of their disruption by chemicals would obviously aid in the development and improvement of insecticidal compounds.

The energy required for life processes is released in cells by the chemical degradation of metabolites in a step-by-step manner through certain well-defined chains of enzymic reactions, some of which represent common pathways, others alternative, more or less independent ones (Barron, 1939; Elvehjem *et al.*, 1939; Green, 1940; Cori, 1942; Meyerhof *et al.*, 1942; Wilson *et al.*, 1942; Dorfman, 1943). These reactions can be interrupted, and hence revealed, by substances known as inhibitors, which, by reason of their chemical structure and affinities, compete with metabolites for chemical or physical union with particular respiratory enzymes, (Quastel, 1930; Bernheim, 1942).

Accordingly, inhibitors are used widely for the tracing of pathways of metabolism. Furthermore, the inhibitory effect is, in general, proportionate to inhibitor concentration and the quantitative relationships can be described by the mathematical expression for the law of mass action (Fisher and Öhnell, 1940; Armstrong and Fisher, 1940; Fisher, 1942; Fisher and Stern, 1942).

If the routes of energy release can be traced by the effects of inhibitors whose specific action is known, then conversely, a knowledge of the pathways of catabolism in an organism should provide a background for the exploration of effects produced by other inhibitors. It would facilitate a more efficient selection and combination of poisons to disrupt the common or alternative routes of energy release which may confer insecticidal resistance upon an insect. Furthermore, the expression of poison-enzyme affinities according to the mass law formula should provide a sound basis for comparison of inherent toxicities of different poisons whose action is on oxidative processes.

The investigation reported on here was undertaken as a contribution to insect toxicology in which the main features of carbohydrate metabolism in certain tissues of an insect are investigated as a basis for revealing qualitative and quantitative differences in the action of different poisons.

MATERIALS AND METHODS

Larvae of the codling moth (*Cydia pomonella*, L.) were chosen for the experiment on account of (a) their availability in large numbers at a convenient time of the year, and the ease of storing them alive in refrigeration ready for use throughout the winter; (b) the extensive background of published information on this insect, which would be available where necessary; (c) the status of this species as a pest of prime economic importance, difficult to control.

The insects were collected in September near Vineland, Ontario. The larvae were segregated according to sex and allowed to spin hibernacula in short rolls of corrugated cardboard in which they were then stored at 7° C., a temperature which is within the optimum range for storage of this insect. About 3,000 insects were used in the experiments.

In order that the separate effects of respiratory inhibitors on particular tissues could be observed, and in order that the concentrations might be regulated accurately, it was necessary to study isolated preparations suspended in a fluid medium. In this way the limitations imposed by diffusion through the insect cuticle or by

cessation of respiratory movements could be avoided. Preliminary experiments demonstrated the necessity of separating the tissues into two fractions, one consisting of fat-body, the other of muscle with hypodermis and other tissues. It was not feasible to make further segregation of tissues since the muscle constituted the greater proportion of non-adipose tissue, and the respiration of the nerve tissue, alimentary canal, and hypodermis could not be measured in the apparatus. In insects the fat-body is more than a mere food-storage tissue, being made up of cells capable of intense activity, particularly during metamorphosis, and therefore requires consideration in the study of poisons. Wigglesworth (1939) points out that the term "fat-body" is a misnomer, for while it contains a large amount of fat, it is equally important as a store for proteins and sometimes for glycogen. In the fat-body of the silkworm, for example, the glycogen is estimated to range from 2 to 17 per cent.

Dissections were carried out with sharp-pointed forceps by slitting the larvae longitudinally along the latero-ventral region of the body. The fat-body substance was then stripped away from the muscles and hypodermis by pressure of the forceps along the operator's forefinger. The isolated preparations were suspended in a measured volume of 0.05 molar sodium phosphate buffer at pH 6.3, the normal pH of blood in codling moth larvae. Potassium salts were avoided because of possible interference specifically due to the potassium ion (Fenn, 1940). Where the presence of intact tissues made pipetting impossible, the volume, 1.80 ml., of tissue and buffer used for each sample was measured in a graduated 15 ml. tapered centrifuge tube which provided the necessary accuracy for such a volume. This amount made possible the addition, during the course of the experiment, of 0.20 ml. of inhibitory or other substances to yield a final concentration equal to one-tenth the strength of the added solution. According to the requirements of a particular experiment, the tissues of from eight to fifteen larvae were used. In some preliminary experiments, macerated tissues, passed through bolting silk, were used, but the intact tissues were employed in later experiments on account of the greater stability of their respiration and the smaller amounts of material required. The intact living organism was used for determination of the normal respiration and respiratory quotient.

The experiments were directed, first, toward tracing, in a general way, the main routes of carbohydrate utilization in order that sensitivity to inhibitors could be explored. These important known pathways of energy release from carbohydrates in cells include:

(1) oxidation of hexose sugars or glycogen, in which oxygen is utilized and carbon dioxide liberated in equivalent amounts; (2) aerobic glycolysis, requiring the presence of oxygen, but not its utilization, to produce carbon dioxide; (3) anaerobic glycolysis, utilizing glycogen in the absence of oxygen, to produce carbon dioxide and other end products. Since these different processes are characterized by different oxygen-carbon dioxide relationships, they can be recognized and measured largely by gas-exchange techniques, supplemented by analyses for metabolite utilization and non-gaseous end products. Oxidation can thus be measured by rate of oxygen uptake, and when not accompanied by the production of glycolytic carbon dioxide, the respiratory quotient (CO_2/O_2) should be unity. Aerobic glycolysis concurrent with oxidation, results in an R.Q. exceeding unity to an extent depending on the relative rates of the two processes, and it can therefore be measured by the R.Q. Anaerobic glycolysis can be measured by the rate of carbon dioxide or lactic acid production anaerobically. As a corollary of the foregoing principles, the effects of inhibitors on catabolism can be observed and measured quantitatively through the same methods.

Rates of oxygen consumption were measured by means of a Warburg respirometer (cf. Dixon, 1943) provided with fourteen flasks and manometers, two of which were used for thermobarometric controls. The amount of respiring tissue in each 1.80 ml. sample was chosen to give a gas pressure change of several millimeters during five-minute intervals. The temperature throughout the experiments was thermostatically maintained at 20° C. Rate of agitation was at 120 times a minute to eliminate surface diffusion as a limiting factor in gas exchange.

Respiratory quotients were determined by measuring first the oxygen uptake, then the net gas pressure change due to the difference between oxygen absorbed and carbon dioxide liberated. Calculations of absolute gas volumes were made according to the methods described by Dixon (1943).

The procedure followed in these determinations of gas exchange for R.Q. determinations was the one employing the same vessel for measuring O_2 consumption as for CO_2 output. With NaOH-soaked paper in the inset of the Warburg flask for absorbing CO_2 , the negative pressure observed gave the measure of O_2 consumption. The observed rate of pressure change, multiplied by the vessel constant at the temperature of the experiment (20° C.) gave the correct absolute value for O_2 consumption. That is, O_2 consumption = observed

$O_2 \times K_{O_2}$. In the calculations for determining CO_2 output this was symbolized in brief, as x . The second stage in the measurements involved the removal of the alkali, including swabbing of the inset with acid, and placing of 0.3 ml. of 3 N HCl in the side arm of the flask. After a series of readings was made, the acid was tipped out of the side arm into the experimental material to drive out any residual CO_2 . A control containing buffer without tissue was similarly acidified to test for any dissolved CO_2 . In the acidification the pH changed from 6.3 to 0.6. Results showed no retention of CO_2 by the buffer at pH 6.3. The measurements gave the net change in gas volume in the flask due to the difference between O_2 consumed and CO_2 produced: $CO_2 - O_2 = y$. The CO_2 produced was calculated from the equation: $CO_2 = (y + x) K_{CO_2}$, K_{CO_2} being the vessel constant for CO_2 at the temperature of the experiment.

Anaerobic conditions in the respirometer for study of anaerobic CO_2 were produced by flushing the vessels with 250 ml. of pure nitrogen. Since the commercial tank nitrogen to be used contained about 0.5 per cent of oxygen as an impurity, it was necessary to de-oxygenate it by passing it through heated copper gauze reduced by hydrogen at $420^\circ C$. Care was taken to avoid readmission of oxygen into the flasks and manometers after flushing with nitrogen. Anaerobic conditions to which larvae intended for glycogen study were subjected, were obtained by chemicals. Living larvae were suspended in a tiny wire gauze cage and sealed in an anaerobic culture jar, the lid of which consisted of a plate culture of luminous bacteria. The oxygen was then absorbed by mixing solutions of pyrogalllic acid and alkali across the barrier in the bottom of the jar. Loss of luminosity of the bacteria during anaerobiosis and gradual return of luminescence on readmission of air provided a sensitive test for the conditions sought.

Glycogen was determined in terms of glucose equivalents by the methods of Elliott and Schroeder (1934) and of Hagedorn-Jensen (Peters and Van Slyke, 1932). Briefly, the method depends on digestion and removal of proteins by KOH, precipitation of glycogen with Na_2SO_4 and discarding of soluble carbohydrates, hydrolysis of glycogen to hexoses and their determination by reduction of potassium ferri-cyanide in an alkaline solution.

The presence of cytochrome oxidase was determined by taking advantage of the specific affinity of carbon monoxide for that respiratory enzyme and the release of the resultant inhibitory action by intense light. The carbon monoxide was generated by dripping 90

per cent formic acid into hot concentrated sulphuric, the reaction consisting essentially of a dehydration process. Formic acid escaping with the CO was absorbed by KOH. A carbon arc, provided with a heat-absorbing filter, was used as a source of illumination (Fisher and Cameron, 1938).

A quantitative measure of the activity of cytochrome oxidase was obtained through the addition of a graded series of concentrations of hydroquinone (cf. Stotz, Sidwell, and Hogness, 1938) until a maximum rate of oxygen consumption was reached.

Cytochrome C was sought for with a Leitz microspectroscope. Since absorption bands by this substance appear only when it is in the reduced form, the tissue was kept in the reduced state by addition of cyanide to prevent reoxidation, and by creation of anaerobic conditions through exclusion of air.

When the potential activity of cytochrome oxidase was shown by hydroquinone, carbohydrate substrates were introduced to determine if any of these were limiting factors. These substances were glycogen, glucose and maltose.

The yellow enzyme was not investigated.

In order to test the autoxidation of ascorbic acid in the presence of the isolated muscle and fat-body of the insect, an initial rate of oxygen consumption was determined with isolated tissues in buffer in the Warburg flask, and 0.2 ml. of 0.1 M ascorbic acid in buffer of pH 6.3 in the onset. After the initial rate was measured, the ascorbic acid was tipped into the tissues and the new rate was determined. Controls were operated with ascorbic acid in the onset, and buffer, but no tissue in the flask. Another set of controls contained tissue but no ascorbic acid.

The inhibitors used were compounds, the action of which is at least partially known from general studies on carbohydrate breakdown, and whose action is due to a single ion. Compounds such as, for example, lead arsenate, were excluded from the present investigation since a complex inhibitory effect would be expected from the superimposition of the inhibitory effect of Pb^{++} and AsO_4^{---} . Sodium salts were convenient because of the absence of specific effects due to the sodium ion. Quantitative studies were made with sodium azide, cyanide, arsenite, fluoride, and iodoacetate, concentrations ranging from 0.00001 M to 0.05 M. The pH, measured electrometrically, was controlled by buffering all fluids. Special precautions were necessary in the maintenance of the correct concentrations of cyanide, owing to its property of distilling from the experi-

mental mixture into the alkali used for CO₂ absorption, unless balanced by cyanide in the alkali. The correct amount of cyanide in the alkali for any given concentration in the tissue was determined from the relationship $\frac{\text{NaCN}}{\text{NaOH}} = 10,000 \text{ HCN}$ (cf. Krebs, 1935). Cyanide solutions were prepared immediately before use, since their strength changes through polymerization in concentrated solutions and hydrolysis in dilute ones.

EXPERIMENTAL RESULTS

1. Rates of respiration

(a) *Living larvae* (Table I)

The rate of respiration of living larvae averaged between 19.2 and 26 cu. mm. per hour at 20° C., the absolute rate being slightly greater for females than for males, due to slightly greater size. The rate in terms of dry weight varied from 403 to 508 cu.mm. per gram per hour, which is comparable with the 413 to 433 determined by Cook (1932) for the termite, *Termopsis nevadense*. A gradual rise in oxygen consumption rate occurred after increased time in storage, until a maximum was reached in March, amounting to 120 per cent of the November rate. The time at which the maximum was reached corresponded with the termination of diapause. The rise in rate conforms with the observations of the other investigators who noted differences in the respiratory rates between diapause and non-diapause (Bodine, 1932; Carothers, 1924; Squire, 1936).

(b) *Intact isolated tissues* (Tables II and III and Graph I)

Oxygen consumption of the isolated tissues was characterized by an initially higher rate which declined to a moderately steady rate within the first forty-five minutes after dissection (Graph I), and continued with but slight decrease even after six hours. The stable rate of muscle respiration was slightly higher for females than for males, and in both sexes remained at a relatively constant level during storage at 7° C. for seven months. Oxygen consumption by fat-body exhibited a progressive increase during storage, to a rate in March of two to three times that which was registered in October. As a result of this absolute increase in respiration during storage, an inversion occurred in the percentages of total respiration attributable to muscle and fat-body respectively (Table III). The sum total of the stable tissue respiration of isolated muscle and fat-body was less than half

the rate determined for the intact living organism. The difference may be accounted for, in part at least, by the fact that the living larvae in the flask were moving actively and therefore placed greater demands for their oxygen requirements than their inactive, excised tissues.

TABLE I

AVERAGE RATES OF OXYGEN CONSUMPTION BY CODLING MOTH LARVAE
AND THEIR TISSUES AT 20°C

(Standard deviations indicated for determinations on isolated tissues)

Nature of Preparation	cu. mm. O ₂ per larva per hour		cu. mm. O ₂ per gm. wet wt. per hour		cu. mm. O ₂ per gm. dry wt. per hour	
	November	March	November	March	November	March
1. <i>Females</i> Living larvae	21.6 (10 larvae)	26.3 (15 larvae)	403	490	1710	2080
Combined respiration of the intact isolated tissues	8.6 (± 1.39) (110 larvae)	12.9 (± 1.47) (24 larvae)	160	240	680	1022
2. <i>Males</i> Living larvae	17.1 (35 larvae)	20.8 (15 larvae)	382	465	1590	1938
Combined respiration of the intact isolated tissues	5.77 (± 0.58) (35 larvae)	11.46 (± 1.41) (35 larvae)	129	256	537	1067

(c) *Macerated tissues* (Graph I and Table IV)

The maceration of muscle and of fat-body resulted in an oxygen consumption rate which was less stable than that of the intact tissues. The respiration of fat-body brei was initially twice as great as it was prior to maceration but fell within an hour to a relatively steady rate equal to one-half the initial velocity and comparable with that of the intact isolated tissue. In muscle brei, the initial rate was also twice that of intact isolated muscle, but fell greatly during the first three

hours to a more stable rate of less than one-eighth the initial velocity. The variability of different aliquots pipetted from the same sample is shown in Table IV.

TABLE II

STEADY RATES OF RESPIRATION, CU. MM. OF OXYGEN PER GM.
DRY WEIGHT PER HR. AT 20°C. pH 6.3

(Standard deviation shown in parentheses)

DATE	FEMALES			MALES		
	MUSCLE	FAT-BODY		MUSCLE	FAT-BODY	
		Rates based on total dry weight	Rates based on defatted dry weight		Rates based on total dry weight	Rates based on defatted dry weight
1943- 1944						
Oct. 25, 29	—	—	—	725 (84)	274 (47.6)	553
Nov. 5, 9	934 (99)	411 (95)	830	804 (41.7)	322 (68)	650
Nov. 17	1116 (66)	451 (151)	910	—	—	—
Feb. 9	—	—	—	798 (113.3)	510 (47)	1031
Feb. 11	936 (98.4)	505 (121.5)	1020	—	—	—
Feb. 15	1169 (95.6)	743 (85.6)	1502	—	—	—
Feb. 18	947 (120)	783 (117)	1582	878 (81.5)	811 (138)	1640
Mar. 1	826 (76)	695 (28.5)	1405	—	—	—
Mar. 14	—	—	—	720 (7.3)	804 (114)	1625
Apr. 14, 15	875 (91.2)	666 (45)	1347	—	—	—

TABLE III

TABLE SHOWING PERCENTAGE OF TOTAL RESPIRATION
ATTRIBUTABLE TO MUSCLE AND FAT-BODY

DATE	FEMALES		MALES	
	Muscle	Fat-Body	Muscle	Fat-Body
Oct. 25, 29	—	—	66.5	33.5
Nov. 5, 9	60.4	39.6	67.2	32.8
Nov. 17	64.75	35.25	—	—
Jan. 12	61.8	38.2	—	—
Feb. 9, 11	51.3	48.7	52.1	47.9
Feb. 15	48.0	52.0	—	—
Feb. 18	37.2	62.8	—	—
Mar. 1	40.0	60.0	43.2	56.8
Mar. 14	38.1	61.9	—	—
Apr. 14, 15	44.6	55.4	—	—

TABLE IV

OXYGEN CONSUMPTION OF MACERATED TISSUES PASSED
THROUGH BOLTING SILK OF 35 MESH PER CM.

(Aliquots of 1.80 ml. equivalent to tissue
of 8 female larvae. Temp. 20°C. pH 6.3)

MUSCLE BREI		FAT-BODY BREI	
cu. mm. O ₂ per hour	Mean value and standard deviation	cu. mm. O ₂ per hour	Mean value and standard deviation
12.06	11.56 ± 1.38	4.53	4.96 ± 0.72
14.00		6.02	
10.36		5.60	
11.88		4.76	
10.45		3.99	
10.62		4.87	

2. *Rates of respiration in relation to oxygen tension* (Table V)

The respiration of living larvae was unaffected by variations of oxygen concentration between 10 per cent and 100 per cent in gas mixtures containing O₂ and N₂. This demonstrated the feasibility of studying the effect of carbon monoxide which must constitute at least 90 per cent of a mixture with oxygen in order to bring about inhibition of respiration (Fisher, 1940). Even in an atmosphere containing only about 1.5 per cent O₂, the oxygen uptake was nearly 20 per cent of normal.

TABLE V
OXYGEN CONSUMPTION AT DIFFERENT OXYGEN CONCENTRATIONS
CU. MM. PER HOUR

(For groups of 5 male larvae at 20° C. Nov. 30, 1943)

Group No.	per cent O ₂	1.5 per cent O ₂	10 per cent O ₂	25 per cent O ₂	100 per cent O ₂
1	0	4.08	21.9	21.9	—
2	0	—	16.5	—	16.6
3	0	—	—	16.9	16.9

3. *Respiratory quotients* (Table VI)

The average rate of carbon dioxide production of the living insect and of its isolated tissues almost invariably exceeded the rate of oxygen consumption, yielding, with but one exception out of thirty-one determinations, R.Q. values greater than unity. The R.Q. for muscle did not differ statistically from that of fat-body, nor was there any change through storage.

TABLE VI
RESPIRATORY QUOTIENTS OF DIFFERENT PREPARATIONS

Experimental Material	Number of observations	R.Q. Mean value and standard deviation
Living larvae	7 determinations, 5 larvae per determination	1.13 ± 0.248
Intact isolated muscle	12 determinations, tissue of 5 to 10 larvae per determination	1.17 ± 0.099
Intact isolated fat-body	12 determinations, tissue of 5 to 10 larvae per determination	1.22 ± 0.185

4. *Anaerobic production of CO₂* (Graph II)

The production of carbon dioxide anaerobically by isolated tissues in an atmosphere of pure nitrogen was appreciably slower than the rate of oxygen consumption aerobically. Just as oxygen consumption aerobically declined, so carbon dioxide production anaerobically fell to a relatively steady rate, which was attained more quickly by fat-body than by muscle.

5. *Anaerobic glycolysis* (Table VII)

During the twenty-four-hour period of anaerobiosis a marked loss of glycogen in both fat-body and muscle was shown by the glucose determinations made after hydrolysing the glycogen.

TABLE VII
TABLE SHOWING GLYCOGEN BREAK-DOWN ANAEROBICALLY
(Glycogen loss expressed in terms of glucose)

Tissue	mg. glucose per 100 gm. dry weight, two determinations for each category		Per cent loss
	Normal condition	After 24 hours anaerobiosis	
Muscle, females	1.83, 2.04	1.05, 1.11	44.2
Muscle, males	3.15, 3.51	1.87, 2.25	38.0
Fat-body, females	4.50, 4.54	3.23, 3.44	25.3
Fat-body, males	4.46, 4.64	3.32, 3.43	28.2

6. *Recovery from anaerobiosis*

Larvae subjected to anaerobic conditions at room temperature for twenty-four hours were quite limp when removed from the anaerobic culture jar but showed slow and feeble muscular activity. Within an hour, individuals of both sexes removed for observation were crawling actively and were still alive and normal three weeks later.

The absence of an oxygen debt mechanism is shown by the fact that tissues deprived of oxygen for ninety minutes failed to show any increased respiration after readmission of air. Instead, the usual tendency to decline in rate occurred during this period (Table VIII).

TABLE VIII

	Steady rate of O ₂ consumption before anaerobiosis cu. mm. per gm. per hour.	Steady rate of O ₂ consumption before anaerobiosis cu. mm. per gm. per hour.
Muscle	685	468
Fat-body	200	100

7. *Inhibition by action of carbon monoxide* (Graph III)

Carbon monoxide caused inhibitions of oxygen uptake amounting to from 25 to 41.8 per cent in muscle, and from 39.3 to 33.5 in fat-body of female larvae. This inhibition was observed to be repeatedly reversible under the influence of intense illumination, thus demonstrating the presence of cytochrome oxidase.

TABLE IX

EFFECT OF HYDROQUINONE ON RESPIRATION OF TISSUES OF FEMALE LARVAE
RATES IN CU. MM. PER HOUR PER GRAM DRY WEIGHT

(*Figures in parentheses indicate the temporarily accelerated rate following addition of H.Q.*)

Molar concentration of hydroquinone	MUSCLE			FAT-BODY		
	Normal rate cu. mm.	Rate established by H.Q.		Normal rate cu. mm.	Rate established by H.Q.	
		cu. mm.	Per cent of normal		cu. mm.	Per cent of normal
0.000001	850	740	87	785	(876) 600	(111.6) 76.5
0.000033	876	986	113	1046	(1216) 858	(116.4) 82
0.0001	923	1040	113	866	(996) 714	(115.1) 82.5
-0.001	836	2000	239	580	(722) 420	(124.6) 72.4
0.01	630	3390	540	785	(820)	(104.5)
0.01	1065	4820	451	650	(650) 420	(100) 64.6
0.02	1330	8780	660	596	(622) 425	(104.6) 71.4
0.05	675	5150	763	750	(750) 398	(100) 53.1
0.10	955	3710	388	750	(936) 460	(125) 61.4

8. *Cytochrome C*

Spectral absorption bands characteristic of reduced cytochrome C could not be detected in native tissues subjected to conditions favouring reduction, such as anaerobiosis or cyanide inhibition of oxidation.

9. *Activity of cytochrome oxidase* (Table IX)

The addition of hydroquinone to the isolated tissues brought about divergent results between muscle and fat-body. The respiration of muscle began to increase immediately, and attained a maximum rate in fifty minutes. A great potential activity of cytochrome oxidase was shown by the 663 per cent increase in respiration produced by 0.05 M hydroquinone.

In fat-body, on the other hand, a slight increase for ten minutes was followed by a sharp inhibition ranging from 18 to 47 per cent.

10. *Effect of added substrates*

The absence of effect on the O_2 consumption of isolated tissues by addition of three common carbohydrates is shown in Table X.

TABLE X

Substrate	Rates of O_2 consumption cu. mm. per gm. dry wt. per hour			
	MUSCLE		FAT-BODY	
	Before addition	After addition	Before addition	After addition
Glycogen .02 per cent	600	600	833	830
Dextrose .01M	632	630	845	845
Maltose	723	720	453	450

This experiment was carried out in view of the results in which the potential activity of cytochrome oxidase in these tissues is much greater than is normally expressed. If failure in the maximum expression of this potential activity were due solely to deficiency in the carbohydrate substrates tested, their addition would have stimulated the respiration to the level attained with hydroquinone. Since they did not, it is considered that they are not limiting factors in the respiration which the cytochrome oxidase present, is capable of carrying.

11. *Effect of tissues on the autoxidation of ascorbic acid (Table XI)*

A dissimilarity between muscle and fat-body was shown in their effects on the autoxidation of ascorbic acid. In the absence of tissue, the autoxidation of ascorbic acid in 0.05M phosphate buffer at pH 6.3, was rapid from the beginning of the experiment. The presence of muscle retarded this process, but did not prevent it entirely, as shown by the gradual rise in oxygen consumption to twice the initial rate after eight minutes. Fat-body prevented the autoxidation entirely, as shown by the fact that the rate of oxygen consumption declined in the manner characteristic of the isolated tissues alone.

TABLE XI
AUTOXIDATION OF ASCORBIC ACID IN PRESENCE OF TISSUES
(Tissues from 8 male larvae per flask in nos. 2-7 inclusive)

Preparation in main part of Warburg flask	Preparation in onset	Initial 10 min. rate cu. mm.	Effect observed
1. Control: 1.8 ml. of buffer pH 6.3	0.2 ml. of 0.1M. AA in buffer	1.27	Addition of AA to give 0.01M caused immediate rise to 50.5 cu. mm. per 10 min.
2. Control: Isolated muscle in buffer	—	5.8	No AA added. Gradual decline to 3.5 cu. mm. per 10 min., after 80 min.
3. Control: Isolated fat-body in buffer	—	9.01	No AA added. Gradual decline to 5.9 cu. mm. per 10 min., after 80 min.
4. Isolated muscle in buffer	0.2 ml. of 0.1M. AA in buffer	6.7	Addition of AA to give 0.01M. Gradual rise to 13.55 cu. mm. per 10 min., after 80 min.
5. "	"	7.5	Addition of AA to give 0.01M. caused gradual rise to 18.5 cu. mm. per 10 min., after 80 min.
6. Isolated fat-body in buffer	"	9.6	Addition of AA to give 0.01M. Gradual decline as in Control No. 3 to 5.8 cu. mm. per 10 min., after 80 min.
7. "	"	9.2	Addition of AA to give 0.01M. Gradual decline as in Control No. 3 to 5.5 cu. mm. per 10 min., after 80 min.

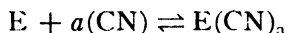
12. *The inhibition of O₂ consumption by inhibitors applied individually*

The quantitative data which are presented in this investigation, pertain to larvae which had terminated diapause as a result of storage for five months at 7° C.

A distinction may be noted between the variability of the determinations of the absolute rates of respiration of any sample of tissues and the error of the determinations of inhibition. Notwithstanding a large standard deviation for the absolute rates of respiration (Table II), the values for percentage inhibition were always closely reproducible, as shown by the distribution of points in the graphs.

The direct relationships existing between the rate of respiration and inhibitor concentration, when expressed graphically, are in the form of rectangular hyperbolae. It is, however, more convenient to express the relationships in terms of the logarithm of inhibitor concentration. These curves, relating rate of respiration, in terms of per cent of normal, to the logarithm of inhibitor concentration, assume sigmoid characteristics (Graphs IV, VI, VIII, X, XII). In all instances a residual respiration remained which was insensitive to even the highest concentration of inhibitor. The amount of this residual respiration varied according to the inhibitor. (Table XII).

The applicability of the mass law principle for the mathematical analysis of the reaction which occurs between an inhibitor and an inhibitor-sensitive component of the oxidative system, was first demonstrated by Warburg (1927), and developed further by Fisher and Öhnell (1940). The application is made on the assumption that the measurable effect (in this instance diminished oxygen uptake), is also a direct measure of the enzyme which has reacted with the inhibitor. The equation is written, in the case of cyanide, for example:



in which E represents the amount of free enzyme, as measured by respiration still functioning; CN represents the free cyanide; and ECN represents the inhibited enzyme, measured by diminution of respiration.

The mathematical expression is then:

$$\frac{[E] \times [\text{CN}]^a}{[E(\text{CN})_a]} = K$$

In logarithmic form, this becomes:

$$\log \frac{[E]}{[ECN]} \cdot a \log [\text{CN}] = \log K$$

A straight-line graph of slope a is obtained when $\log \frac{[E]}{[ECN]}$ is plotted against $\log [CN]$. In practice, $\frac{[E]}{[ECN]}$ is represented by the experimentally measured: $\frac{\% \text{ Uninhibited}}{\% \text{ Inhibited}}$, written simply in the form $\frac{U}{I}$. Since residual fractions of respiration occurred, which were unaffected even by the greatest concentrations of inhibitor, these residual values represented reactions which were not concerned in the equation relating to inhibition. They were, consequently, subtracted from the observed values to yield the true value for the uninhibited respiration concerned.

In the present experiments the curves are, with two exceptions, to be noted later, doubly sigmoid, showing a discontinuity of effect in the intermediate range of inhibitor concentrations. Moreover, this discontinuity is accompanied by a slight rise in respiratory rate as the inhibitor concentration increases through a relatively narrow transition zone. After the slight rise, the rate of respiration again falls, to reach a final residual level. This phenomenon has its analogy in the results of Yeager and Munson (1944) who found an increase in survival time of cockroaches injected with sodium metarsenite as the concentration of this poison passed from 0.03 to 0.04 molar. This was followed again by a decline in survival time. Analyses of the data in the present investigation were made on the assumption that the anomalous effect did not invalidate the fundamental postulate of the applicability of the mass law principle. The justification for this assumption is considered in the Discussion. The straight line relations between the logarithm of inhibitor concentration and logarithm of $\frac{U}{I}$ are shown in Graphs V, VII, IX, XI, and XIII. Since the points which correspond to the hump in the percentage inhibition curves are believed to represent the superimposition of a secondary effect on the main inhibition curves, these could not be used to give weight to the placing of the straight line through the other points. The numerical coefficients derived from the mass law expressions are presented in Table XII. The significance of these values is considered in the Discussion.

(a) *Sodium azide* (Graphs IV and V)

【Inhibition of respiration in both muscle and fat-body occurred throughout the range of azide concentrations used (0.00001 to

0.025M), and was, in general, proportionate to them. The fat-body respiration, however, was affected to a greater degree, especially with the intermediate inhibitor concentrations. A pronounced discontinuity of effect is evident for both types of tissue at an azide concentration nearing 0.001 M. Moreover, this discontinuity is accompanied by a slight rise in respiratory rate as the azide concentration increases from 0.001 to 0.002 M., from which point the rate again declines. With the exception of the points showing what is believed to be a reactivating effect in this region of the curves, the general relations between azide concentration and rate of O_2 consumption conform to the mathematical relationships of the mass law.

(b) *Sodium cyanide* (Graphs VI and VII)

The effect of cyanide on respiration of both muscle and fat-body was similar to, though slightly greater than that of, azide. Inhibition occurred throughout the range of concentrations, (0.00001 to 0.05 M), and in proportion to them, the effect on fat-body again being greater than on muscle. A discontinuity of effect is shown in the curves at cyanide concentrations near 0.003 M. Respiration was not completely inhibited even by 0.05 M cyanide. General conformity of the data with the mass law formula is shown by their resolution into straight line relationships.

(c) *Sodium arsenite* (Graphs VIII and IX)

The respiration of both muscle and fat-body was inhibited throughout the range of arsenite concentrations between 0.00001 and 0.05 M. As with the previous two compounds, a discontinuity of effect occurred near 0.001 M., but whereas the respiration of muscle rose slightly and then fell with increased inhibitor concentrations, that of fat-body continued to rise rapidly back towards the normal rate.

(d) *Sodium iodoacetate* (Graphs X and XI)

Respiration is inhibited progressively throughout the concentration range 0.00001 to 0.05 M. Again that of the fat-body is more sensitive than that of muscle, but whereas the curve for inhibitor action on muscle is compound, the one for fat-body is of simple sigmoid form.

(e) *Sodium fluoride* (Graphs XII and XIII)

The inhibitory effect of fluoride on oxygen consumption by muscle and fat-body is pronounced, but considerably less so than that of

azide or cyanide. A discontinuity of effect is again observable but this time near a concentration of 0.005 M for both types of tissue. Fat-body respiration is somewhat more sensitive to fluoride than is that of muscle.

TABLE XII

THE COEFFICIENTS DERIVED FROM THE APPLICATION OF THE
MASS LAW EQUATION TO THE INHIBITION CURVES

INHIBITOR	INTACT ISOLATED MUSCLE			INTACT ISOLATED FAT-BODY		
	Per cent residual allowed for	<i>a</i>	K	Per cent residual allowed for	<i>a</i>	K
Sodium azide...	20	0.78	5.50×10^{-3}	16	1.02	2.40×10^{-4}
Sodium cyanide...	22	1.05	5.25×10^{-6}	15	1.10	1.27×10^{-5}
Sodium arsenite...	66	0.97	1.57×10^{-3}	47	1.05	2.70×10^{-5}
Sodium iodoacetate	52	0.475	1.33×10^{-2}	15	0.50	7.76×10^{-3}
Sodium fluoride...	69	1.00	2.51×10^{-3}	30	0.97	3.16×10^{-3}

13. *Combined effects of inhibitors*

A comparison between the effects of inhibitors used individually and in combination with one another may be seen in Table XIII.

In all combinations tested except one (*) there was no significant summation of effects, the inhibition caused by the combination being no greater than that contributed by the more strongly inhibitory member of the pair. The mixture of 0.001 M arsenite and 0.010 M fluoride, provided the exception in which a partial summation of effects occurred.

14. *Effect of inhibitors on R.Q.*

The introduction of the respiratory inhibitors, fluoride, cyanide, and iodoacetate to the respiring intact isolated tissues results in a marked rise of R.Q. even with the lowest concentrations used, the

increase being greater with fat-body than with muscle (Table XIV). This expression of a differential effect on the two members of the ratio: $\frac{\text{CO}_2 \text{ produced}}{\text{O}_2 \text{ consumed}}$ is more pronounced with cyanide than with the other two inhibitors, revealing a relatively greater suppression of O_2 uptake than of CO_2 production. Fluoride and iodoacetate, on the other hand, tend to depress the CO_2 production and O_2 consumption to a more nearly equal degree.

TABLE XIII

Inhibitor Combinations	Inhib. Conc's.	Per cent inhibition of muscle respiration		Per cent inhibition of fat-body respiration	
		Inhibitors acting separately	Inhibitors acting in combination	Inhibitors acting separately	Inhibitors acting in combination
Sodium azide . . .	0.001 M	37	35	64	60
Sodium fluoride . . .	0.001 M	11		18	
Sodium arsenite . .	0.001 M	30	32	52	50
Sodium fluoride . . .	0.001 M	11		18	
Sodium arsenite . .	0.001 M	30	41*	52	73*
Sodium fluoride . . .	0.010 M	20		35	
Sodium iodoacetate.	0.001 M	30	35	65	68
Sodium fluoride . . .	0.001 M	11		18	
Sodium arsenite . . .	0.001 M	30	53	52	80
Sodium iodoacetate.	0.050 M	51		81	

15. *Inhibition of anaerobic glycolysis*

Only partial susceptibility of anaerobic glycolysis to inhibition by fluoride is shown in the decline of anaerobic CO_2 production when .01 M sodium fluoride is applied to muscle or fat-body:

TABLE XIV

TABLE SHOWING RELATIONSHIP BETWEEN INHIBITION AND RESPIRATORY QUOTIENTS

(Each figure represents the average R.Q. for the tissues of eight larvae)

Inhibitor Concentration (Molar)	Intact Isolated Muscle			Isolated Fat-Body		
	Before inhibitor added	After inhibitor added		Before inhibitor added	After inhibitor added	
		Per cent inhibition	R.Q.		Inhibition	R.Q.
Sodium cyanide						
0.0005	1.15	68	2.14	1.20	77	3.04
0.001	1.15	72	1.53	1.15	78	2.48
Sodium fluoride						
0.000001	1.11	11	1.45	1.08	4	1.58
0.00001	1.05	12	1.30	1.07	5	1.26
0.00003	1.20	12.5	1.24	1.15	8	1.33
0.05	1.16	35	1.43	1.20	66	1.76
Sodium iodoacetate						
0.00003	1.25	21.5	1.44	1.18	38.2	1.55
0.0001	1.18	24	1.43	1.22	44	1.83
0.001	1.24	29	1.30	1.29	60.3	1.75
0.01	1.24	31	1.52	1.20	71.5	1.73
0.05	1.22	46.5	1.25	1.67	76	1.70

TABLE XV

	AVERAGE RATES OF CO ₂ PRODUCTION (cu mm/gm per hour)	
	MUSCLE	FAT-BODY
Before addition of fluoride	153	80
With 0.01 M fluoride	92	53

DISCUSSION

The free energy required by a cell for its life functions is derived from foodstuff molecules by two methods, fragmentation and combustion. The first method is referred to as fermentation, or glycolysis, the second as oxidation (Szent-Györgyi, 1938). These two processes differ both in regard to their mechanisms and their efficiency for releasing energy, oxidation freeing many times as much energy from a foodstuff molecule as does glycolysis. Moreover, the functioning of the oxidative process suppresses that of glycolysis, in the manner of the Pasteur Reaction (Dixon, 1937).

The relative independence of oxidation and glycolysis suggests that an organism possessing both mechanisms would normally derive most of its energy through the economical and highly efficient method of oxidation. If, however, it is deprived of oxygen, it would survive by obtaining energy glycolytically until aerobic conditions were restored or until the organism exhausted its food reserves by this extravagant method, or until accumulation of fermentation products caused autointoxication.

The occurrence of the two methods of carbohydrate utilization in larvae of the codling moth has been shown manometrically in these experiments. The existence of a respiratory quotient of approximately 1.2 indicates that for every five molecules of oxygen consumed, six molecules of carbon dioxide are released. Since oxidation of a carbohydrate should involve equivalent amounts of O_2 and CO_2 ,



the additional CO_2 molecule, shown by the expression: $\frac{5+1}{5} = 1.2$,

must have originated glycolytically. This would indicate that aerobic glycolysis proceeds slowly in the presence of oxidation. If the Pasteur Reaction does occur in these tissues, then the inhibition of oxidation should result in accelerated glycolysis. The actuality of this is demonstrated in the following manner.

Let us consider, for example, the effect of 0.0005 M cyanide on the R.Q. of fat-body (Table XIV). Here, when respiration is reduced to 23 per cent of normal, the R.Q. rises from 1.20 to 3.04. Prior to inhibition the gas-exchange is made up of the following components:

oxidation.....	5 $O_2 \equiv$ 5 CO_2	
glycolysis.....	— 1 CO_2	
Total.....	5 $O_2 \equiv$ 6 CO_2	R.Q. = 1.20

When oxidation is inhibited to 23 per cent by cyanide, if no other changes occurred, the hypothetical relationships would be as follows:

$$\begin{array}{rcl}
 \text{oxidation....} & 1.15 \text{ O}_2 \equiv & 1.15 \text{ CO}_2 \\
 \text{glycolysis} \quad \dots & \text{—} & 1.0 \text{ CO}_2 \\
 \hline
 \text{Total} \quad \dots & 1.15 \text{ O}_2 \equiv & 2.15 \text{ CO}_2 \quad \text{R.Q.} = 1.87
 \end{array}$$

Since, however, the R.Q. actually rose to 3.04, the discrepancy between that figure and the calculated must have been derived through acceleration of glycolysis when the oxidative process was inhibited.

The sufficiency of the glycolytic process for maintaining life temporarily, is demonstrated in the survival of larvae for twenty-four hours under strictly anaerobic conditions. This phenomenon in insects was also demonstrated by Cook (1932) who recorded recovery of termites after two days of anaerobiosis. The existence of this power of anaerobic survival, coupled with the acceleration of glycolysis during cyanide inhibition of oxidation, suggests a possible mechanism for insect recovery from paralytic but sub-lethal doses of certain poisons. In this process of recovery, glycolysis could maintain life until the inhibitory action is reversed by excretion or other disposal of the inhibitor. Reversal would be in accordance with the mass law principle: $E + I \rightleftharpoons EI$ where E represents the respiratory enzyme concerned, I, the inhibitor, and EI, the enzyme-inhibitor complex. The application of the foregoing principles to insecticide preparation leads to the proposition that the simultaneous retardation of both glycolysis and oxidation by an insecticide would be required for the highest insecticidal power.

The discontinuity in the inhibitor effect in the intermediate region of the concentrations employed, and the apparent displacement of the points in the higher inhibitor concentrations at first suggested the beginning of inhibition of a second enzyme after an end point for a first enzyme was approached. If such were the case, the effect could have been regarded as analogous to titration curves for dibasic acids or mixtures of two acids titrated with a base, and the inhibition curves could then have been broken into separate sigmoid components and these individual parts analysed as independent entities. There was, however, no other evidence to support the assumption that the observed effect was due to the inhibition of more than a single enzyme. Furthermore, the fact that different final inhibition levels were established under the influence of the different inhibitors, indicated that the enzymes inhibited were not the same with all inhibitors, and could not all be expected to exhibit the same unusual effects shown in the

curves. Moreover, the assumption of two enzymes, inhibited successively by the same inhibitor, could not explain the existence of the rise in the region of discontinuity.

The anomalous effect appears to be more consistent with the occurrence of critical solute concentrations which result in a negative adsorption effect in certain colloidal systems. In this process a concentration of the solvent rather than of the solute occurs in the interfacial film on the adsorbent after a certain amount of solute is added to the solution (Thomas, 1934). This effect is also manifested in osmotic effects produced by many electrolytes. With such substances, osmosis falls off at increased concentrations and begins to increase only at still greater ones as required by augmented osmotic pressure of the solution (Freundlich, 1925). It appears reasonable that this phenomenon may be related to the dissociating effects of solutes on the hydrol aggregations of water (cf. Bousfield and Lowry, 1910).

In the inhibition of the enzymes concerned in the present experiments, the preferential adsorption of water as compared with adsorption of inhibitor on the enzyme surface would, in effect, cause a dilution of the inhibitor in contact with the enzyme. This would result in a lower degree of inhibition than might be expected from the inhibitor concentration in the solution. A rise in the inhibition curve would follow. Since negative adsorption is always small in amount, and in most cases only a monomolecular film of solvent is adsorbed at the interface (Gortner, 1938), the amount of water adsorbed would quickly attain a maximum. As the inhibitor concentration increased, inhibition would again be resumed and the curve would fall from the hump.

This assumption of a common physico-chemical phenomenon seemed to be compatible with all the observed data. It provided a common explanation which could most plausibly account for the hump in the curves for diverse inhibitors. Accordingly, each inhibition curve was treated as a single entity rather than as two successive ones. The final residual level where the curve became asymptotic with the abscissa, was used as the value to subtract from observed rates to give the true value for U in the mass law equation.

In considering the effect of an inhibitor on R.Q. several combinations of inhibition of oxidation and glycolysis might occur. In a system in which both oxidative and glycolytic processes occur, the inhibition of oxidation alone would cause a drop in O_2 uptake and a corresponding drop in *oxidative* CO_2 output; *glycolytic* CO_2 would

remain constant and a rise in R.Q. would result. By similar reasoning, it follows that in such a system the inhibition of glycolysis alone would cause a fall in R.Q. If, however, both processes are inhibited, the effect on R.Q. will depend on any differential action which may occur between the inhibition of oxidation and glycolysis. A relatively greater inhibition of oxidation than of glycolysis would cause the R.Q. to rise, but not to the extent which would be calculated on the basis of inhibition of the oxidative process alone.

With fluoride, the 66 per cent inhibition of fat-body oxidation by 0.05 NaF, caused the R.Q. to rise from 1.20 to 1.76. By the same calculations as used above for cyanide, 66 per cent inhibition of oxidation alone would cause the R.Q. to rise to 1.59. If in addition, glycolysis were affected, R.Q. would be less than 1.59. A similar slight rise above the calculated was experienced by muscle R.Q. In this tissue, 35 per cent inhibition caused a rise from 1.16 to 1.43 as compared with a calculated 1.25. These results seem to imply fluoride insensitivity of the aerobic glycolysis in these tissues.

With 0.05 M iodoacetate, the 46.5 per cent inhibition of muscle respiration resulted in no essential change in R.Q. determination from 1.22 to 1.25 as compared with a calculated 1.41 (assuming for argument no inhibition of glycolysis). The R.Q. values of fat-body before and after 76 per cent inhibition of respiration were, respectively, 1.67 and 1.70 as against a calculated 2.04. These exemplify the condition in which oxidation and glycolysis have been inhibited at an approximately equal rate.

The absence of an oxygen debt mechanism in the larvae studied is shown by the lack of any compensatory acceleration of oxygen consumption following return from anaerobic to aerobic conditions. Similar lack of oxygen debt in insects was shown in termites by Cook (1932). This characteristic has certain implications from the point of view of insect toxicology. Implicit in the phenomenon is the absence of restoration of food reserves from the cleavage products of fermentation, since restoration requires oxygen. If inhibition of respiration persists until food reserves are wasted away by glycolysis, recovery could not occur even when aerobic conditions are restored. The most lethal inhibitor of biological oxidation would thus be one whose complexes with respiratory enzymes exhibit the lowest degree of reversibility so that its toxic effects would persist.

Certain general principles pertaining to the toxicity of poisons applied jointly have been described by Bliss (1939). From dosage-mortality data he determined three types of effects, classified on the

basis of diversity, similarity, and interaction. Other studies on interaction, leading to synergism or antagonism have been made by Johnson, Eyring, and Kearns (1943), Erichsen Jones (1938), Roark (1944). In the present study, various combinations of inhibitors, in all but one instance, resulted in an inhibition of respiration no greater than the effect of the more inhibitory component (Table XIII), suggesting an action in which the constituents act independently and similarly.

The combination, 0.001 M arsenite and 0.01 M fluoride, appears to represent the independent and diverse action of the two inhibitors. Here it should be noted that the stronger inhibitor is employed in the weaker concentration; at equivalent concentrations the effect was no greater than that of the more inhibitory substance, arsenite.

In the practice of combining insecticides, consideration should thus be given not only to the constitution of chemicals *per se*, but also to their relative concentrations.

The high potential activity of cytochrome oxidase in muscle was shown by the rapid oxidation of hydroquinone. In view of the inhibition produced by carbon monoxide, which showed the presence of cytochrome oxidase, the failure of fat-body to oxidize hydroquinone must be accounted for by a reason other than absence of cytochrome oxidase. The failure in oxidation of hydroquinone by fat-body suggested rather the existence of a strongly reducing substance in the fat-body. This belief was supported by the fact that the addition of l-ascorbic acid led to a rise in oxygen consumption by muscle but not by fat-body. It was noted, however, that in contrast with controls of ascorbic acid in buffer, the muscle also had considerable restraining effect on the oxidation. One possible mechanism of the restraining action on these oxidations would be reduction by glutathione which is commonly found in insects (Fink, 1927). Such a protective action of tissue extracts in preventing the destruction of AA was explained by Mawson (1935), as being due, in part at least, to their content of glutathione (Crook, 1941).

The high degree of inhibition produced by azide and cyanide on both fat-body and muscle, strengthens the belief that cytochrome oxidase occupies a key position in the oxidative processes in this insect.

Since cytochrome oxidase evidently plays a dominant role in respiration of this insect, it appears reasonable that poisons of metal catalysis other than azide or cyanide would be strongly inhibitory. The quantitative effects shown by azide and cyanide would serve as a basis for comparisons.

The divergence in the effects of sodium arsenite on fat-body and muscle respiration at concentrations above 0.001 M at once emphasizes a dissimilarity in their respective responses to this inhibitor. The curve for muscle follows a pattern somewhat similar to that of azide and cyanide but with smaller maximum inhibition. The discontinuous process of inhibition is again evident, with a slight recovery of respiration following inhibition of the first component.

The remarkable return of fat-body respiration toward normal as the concentration of arsenite is increased beyond 0.001 M, draws attention to another distinction between fat-body and muscle. One condition in which an arsenical compound causes acceleration of carbohydrate break-down was investigated by Harden (1930). When the rate of fermentation is controlled by the rate at which inorganic phosphate is supplied by hydrolysis of phosphoric esters present in the system (by the phosphatase also present), arsenate accelerates the fermentation by greatly stimulating the effect of the enzyme phosphatase. In the present investigation, however, the phosphate buffer in which the tissue was suspended ensured a large surplus of inorganic phosphate. The curve for fat-body respiration suggests rather that the same process which causes a hump in the other curves is exaggerated in fat-body. Since the concentrations of all inhibitors used were kept within the hypotonic range to avoid the intervention of any possible osmotic effects by plasmolysis, the effects of arsenite concentrations greater than 0.05 M are not known.

The region of discontinuity of inhibitor effect shown in the curve near 0.001 M corresponds, in the case of sodium arsenite, to the lethal dose determined by Haseman and Meffert (1933). These investigators found that a dosage of 0.00275 mg. NaAsO_2 injected into the haemocoel of codling moth larvae, killed 85 to 90 per cent in four hours. In the present investigation it was determined that the average mature larva would contain about 37.3 mg. of free water. The concentration resulting from the 0.00275 mg. NaAsO_2 would be 0.00057 M. The logarithm of this is -3.146 , and is of the same order of magnitude as -3.000 , the logarithm of 0.001 M.

The inhibition curves for iodoacetate show a divergence of effect between fat-body and muscle. In muscle respiration, a discontinuity of effect again occurs in the region of 0.001 M. With fat-body, however, this discontinuity of effect was not observed.

The general character of the curves for fluoride on both fat-body and muscle resembles the form shown for azide and cyanide but the region of conspicuous discontinuity in effect has shifted close to 0.01 M.

The initial part of the curve shows that the respiration is able to withstand relatively stronger concentrations of fluoride than of the other inhibitors. The high toxicity claimed for fluoride on this insect can scarcely be accounted for by the inhibition of oxidation. Its effect might more reasonably be sought in its inhibition of muscle glycolysis, resulting in paralysis. On the contrary, the high insecticidal action of cyanide can be accounted for by its inhibitory effect on the oxidative mechanism, particularly cytochrome oxidase. The ready reversibility of cyanide inhibition could account for the recovery of insects from sub-lethal paralytic doses of cyanide if such insects possessed a sufficiently active glycolytic mechanism.

The demonstration of the nature of the inhibition curves and their resolvability in a general way into mathematical expressions by application of the mass law equation, brings up the consideration of the meaning of the constants and their applicability to insect toxicology. The value K is a mathematical expression for the affinity existing between the enzyme and the inhibitor. The greater the affinity, the smaller is the value for K . The application of this principle to insect toxicology makes possible comparisons not restricted by an all-or-none measurement such as mortality.

The value a expresses the actual ionic equivalent required to inhibit the enzyme. It is therefore not a measure of affinity, but indicates rather the progression of inhibition with increasing concentrations of inhibitor. From examination of the graphs it may be seen that if the value a , represented by the slope of the mass law plots, is small, inhibitor effect is exerted over a wider range of concentrations than if a is large. The significance of the value a in an insecticide must therefore be interpreted with reference also to the absolute inhibitor concentrations.

SUMMARY OF EXPERIMENTS

Investigations were carried out to explore the means by which an insect obtains energy for life, and as an aid to insect toxicology, to observe the manner in which chemicals may disrupt these functions.

The chief respiratory characteristics of mature overwintering codling moth larvae, and isolated preparations of their muscle and fat-body, were investigated as a basis for measuring the qualitative and quantitative effects of inhibitors of biological oxidations.

Aerobic oxidation, aerobic glycolysis, and anaerobic glycolysis were investigated through manometric measurements of aerobic and anaerobic gas-exchanges. Observations on anaerobiosis were supple-

mented by survival tests and glycogen analyses of larvae subjected to anaerobic conditions for twenty-four hours. The presence and activity of cytochrome oxidase in the aerobic oxidation system was studied through the inhibitory effects of carbon monoxide and azide, and through the accelerating effect of hydroquinone.

The qualitative and quantitative effects of azide, cyanide, arsenite, fluoride, and iodoacetate on oxidation were measured through decreases in oxygen consumption caused by these inhibitors in a series of concentrations from one-twentieth to one hundred-thousandth molar. Resolution of the quantitative effects was made by application of the mathematical expression for the law of mass action in conformity with the principle shown for other biological material by other investigators.

The effects on oxygen consumption of combining certain inhibitors were tested to determine possible additive effects caused by inhibitors acting on alternative routes in the oxidative mechanism.

The effect of inhibitors on aerobic glycolysis was noted through changes in the respiratory quotient.

Inhibition of anaerobic glycolysis was noted through restricted CO_2 output.

The significance of these results in relation to insect toxicology is discussed.

CONCLUSIONS

1. The average rate of oxygen consumption of mature overwintering codling moth larvae ranged from 382 to 490 cu. mm. per gram live weight per hour at 20°C ., the higher rates occurring after four months' storage at 7°C .

2. The greater proportion of the total respiration was accounted for by the respiration of fat-body and muscle. During storage of diapause-larvae at 70°C ., fat-body respiration increased two to three-fold, this change coinciding with the termination of diapause.

3. The combined respiration of intact isolated preparations of muscle and fat-body in 0.05 M phosphate buffer of pH 6.3 at 20°C ., was initially similar to the respiration of the living organism, but during the first forty-five minutes after dissection, declined to about one-half the normal rate. This relatively stable respiration continued with only slight decrease even after six hours.

4. Macerated tissues possessed a less stable respiration than did intact excised tissues, and consequently were less suitable for inhibitor studies.

5. Respiration of living larvae was unaffected by variations in oxygen tensions between 10 per cent and 100 per cent. In an atmosphere containing only 1.5 per cent O_2 , the oxygen uptake was nearly 20 per cent of normal.

6. Respiratory quotients of the living larvae and their isolated muscle and fat-body averaged between 1.13 and 1.22, and demonstrated the occurrence of aerobic glycolysis.

7. Anaerobic processes were recognized by (a) production of CO_2 anaerobically by both muscle and fat-body, (b) survival of larvae subjected to anaerobic conditions for twenty-four hours, (c) 25 to 44 per cent loss of glycogen during twenty-four hours' anaerobiosis.

8. Oxygen debt did not occur in tissues subjected to anaerobic conditions.

9. Cytochrome oxidase was shown by the inhibitory action of carbon monoxide and the reversibility of this inhibition by light. Its potential activity in muscle was indicated by an increase of 663 per cent on addition of 0.02 M hydroquinone. Its potential activity in fat-body was probably concealed by strongly reducing substances. The difference between potential oxidation rate and actual was not due to deficiency of dextrose, maltose, or glycogen *per se*. The major role of cytochrome oxidase in respiration was shown by the fact that about 80 per cent of the oxidative process was inhibited by azide and cyanide.

10. Cytochrome C could not be demonstrated spectroscopically in native preparations of the tissues.

11. Respiration was inhibited in varying degrees by azide, cyanide, arsenite, fluoride, and iodoacetate, that of fat-body being more sensitive than that of muscle. A residual, insensitive fraction which was not investigated, remained.

12. Inhibition was, in general, proportionate to the logarithm of inhibitor concentration. With the exception of iodoacetate on fat-body, the inhibition was observed to occur in a discontinuous manner as shown by a discontinuity in the curves relating inhibitor concentration to effect. The inhibition of fat-body respiration by arsenite progressed until a concentration of about 0.001 M was reached, when additional arsenite restored the respiration to normal. The critical concentration for arsenite corresponds with a lethal dose in the organism.

13. The inhibition curves can be analysed by the application of the mass law equation. The constants so derived provide a mathematical expression of toxicity of a poison on the oxidative process.

14. Respiratory quotient measurements showed that glycolysis is accelerated by cyanide inhibition of oxidation. This would provide a compensatory mechanism for the insect to obtain energy glycolytically while subjected to an inhibitor relatively specific to oxidative enzymes. Aerobic glycolysis was not inhibited by 0.05 M fluoride, and anaerobic glycolysis was only partially inhibited. Oxidation and aerobic glycolysis were inhibited at an equal rate by iodoacetate.

15. In consideration of the existence of residual levels of respiration which were insensitive to maximal concentrations of individual inhibitors, it is believed that the combination of two or more inhibitors, each acting on the different parallel enzyme systems by which oxidation occurs, could arrest oxidation completely. The inhibitors studied here, evidently act on a common pathway of catabolism, since their effects are not additive.

16. In consideration of the occurrence of the three types of carbohydrate catabolism, and the survival value of glycolysis when oxidation is prevented, it is believed that increased insecticidal action may be achieved by preparations which will simultaneously inhibit oxidation, aerobic glycolysis, and anaerobic glycolysis.

17. Since these investigations concern the poisoning of oxidative processes, the procedure herein described would not apply to such chemicals as may disrupt synaptic transmission and other nervous functions which are not directly dependent on oxidation.

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PLATE I

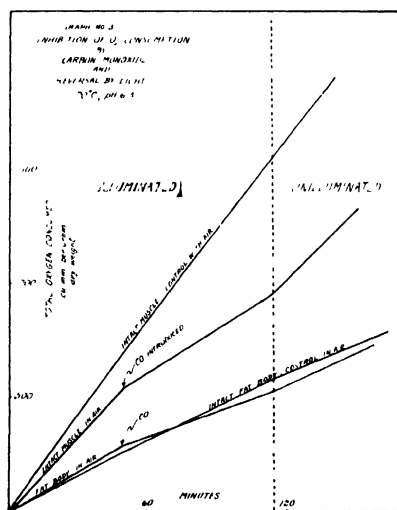
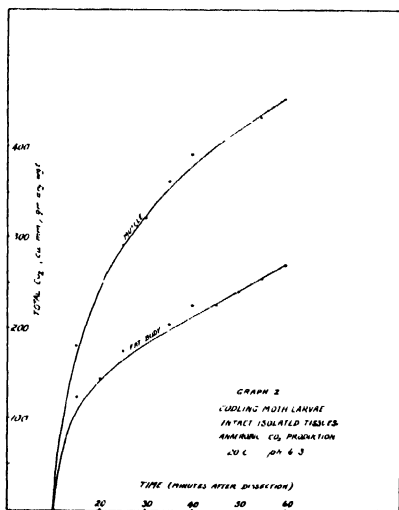
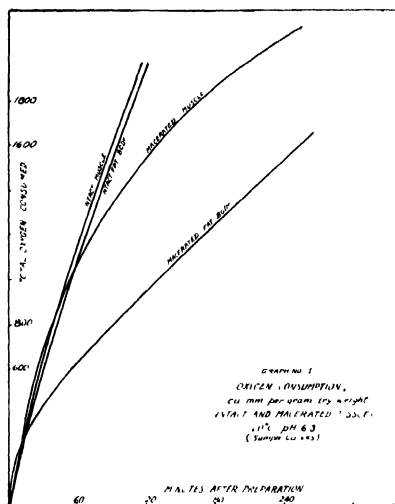


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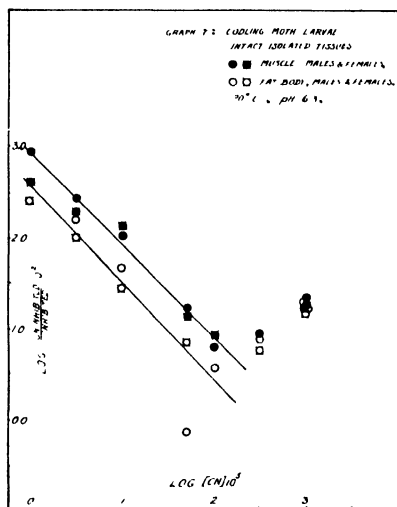
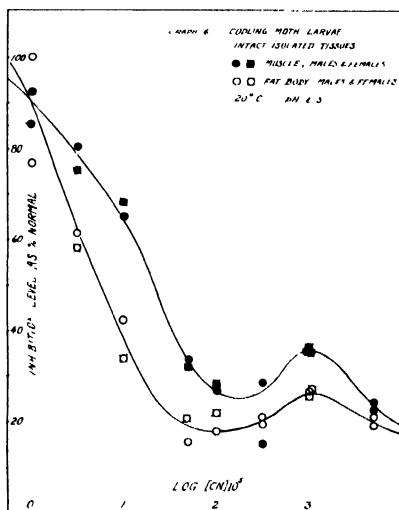
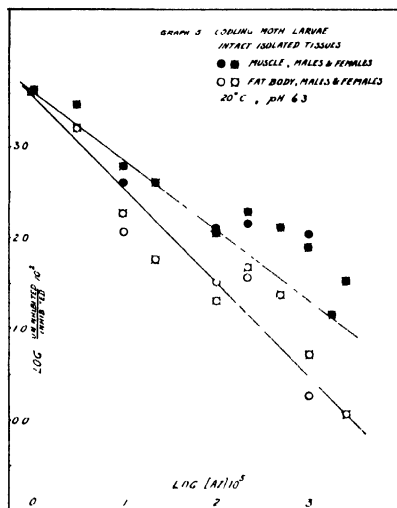
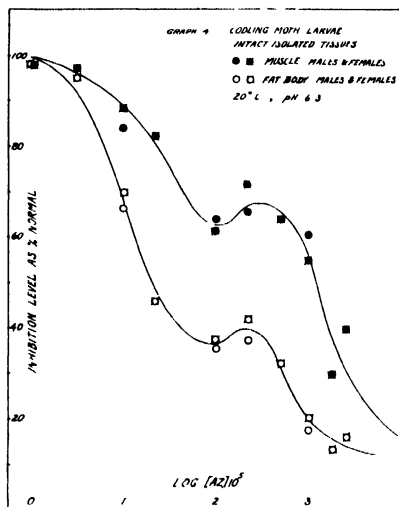


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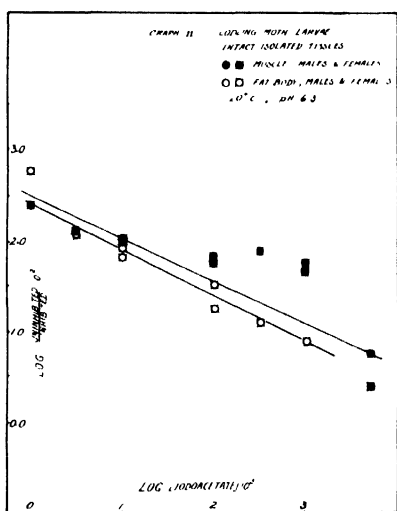
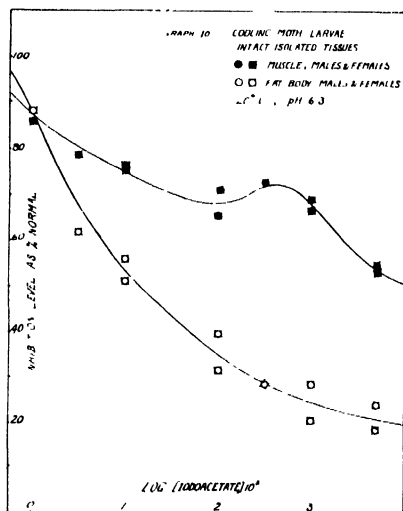
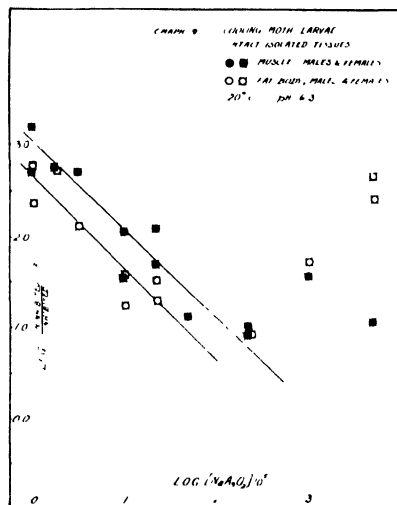
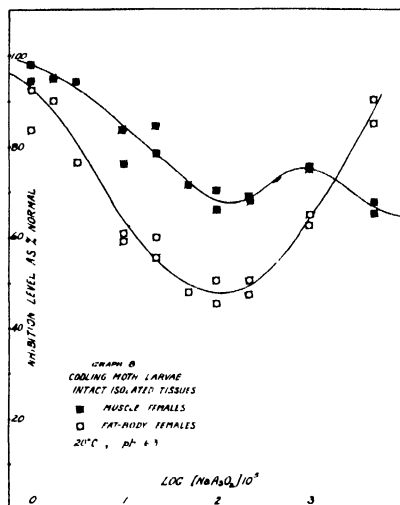
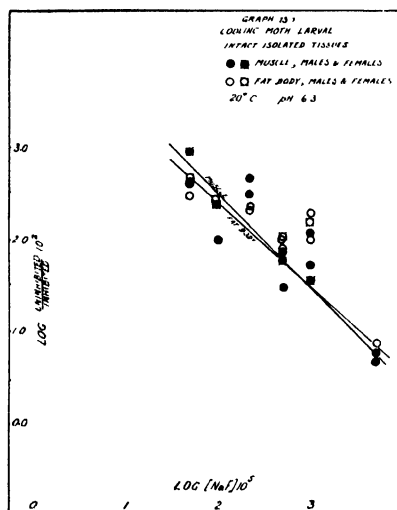
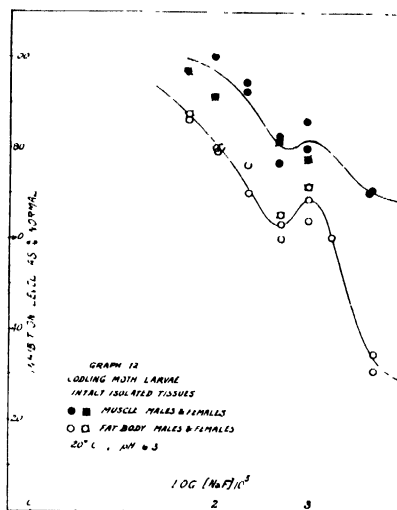


PLATE IV



RANDOM SAMPLING, PLANNED SAMPLING, AND SELECTIVE SAMPLING: AS APPLIED TO FOREST ECOLOGY AND SILVICULTURE

By A. H. HUTCHINSON, F.R.S.C., and F. M. KNAPP

ORDINARILY mathematical statistics is based on the premise or assumption of the validity of "random sampling": that the law of chance results in the normal distribution curve; that the error is reduced as the statistical number is increased; and that exactness is approached, but never reached, at infinity. In practice, observations are discrete and frequently the error is greater at both extremes of an extended series. Recent investigations have led to the conclusion that strictly random sampling does not occur in biological phenomena (Dubinin and Romaschoff, 1932; Fisher, 1928, 1930; Wright, 1940); in other words, a degree of control and direction, in the form of selection, is regularly present; this is another way of expressing the fact that life cannot exist apart from an environment—which seems obvious. To shoot arrows at seedlings while blindfolded would be an attempt at random sampling of forest stocking. This method is not advocated.

Planned sampling recognizes pattern and assumes that the pattern has a crystal-like symmetry. The "Strip Survey Methods" of sampling and the "Line Plot Surveys" are included in this method of analysis. If the strips run north, south, east, west, it is assumed that the primary ecological axis parallels the geographical axis. Over wide areas the geographical axis may give expression to a temperature relationship, while locally a "curved contour axis" is frequently more significant. In the "Stocked Quadrat Methods," a geometrical pattern is relied upon in spite of the fact that the measure is dependent upon a biological complex, specifically the factors which insure a stocking sufficient to provide complete reforestation. This method is limited to significant data on the degree of occurrence as of restocking on a specific area, or areas, and provides no causal relationships or values.

Selective sampling recognizes pattern which is governed by variable conditions and often by a complex of many variables. Selective sampling aims to establish *relationships* between phenomena and the *controlling factors*—not mere occurrences. The axes of pattern may be straight or curved, or variable; there may be one axis, several, or many, according to the number, direction, and values of the forces which are operative. Stocking may be radial as from a tree centre, or it may emanate from a straight or curved forest front, according to the direction of the

wind. Seed germination and seedling establishment may follow soil or humidity, light or other contours. The pattern of selective sampling takes account of ecological contours and recognizes causal, in contrast to random, distribution. An evaluation of causal relationships gives a rational basis for regulation and control which are primary considerations in silvicultural practice. In the words of R. A. Fisher as quoted by Huxley (1945): "Natural selection is a mechanism for generating improbability of a high order." Reasoned selection or "selective sampling" is a correspondingly rapid method of approaching a valid conclusion.

SOME PRACTICAL SILVICULTURAL CONCLUSIONS

1. "Random sampling" has value where there is one or two simple variables, but is not practicable where multiple, variable ecological factors are operative, for instance the factors upon which the restocking of a forest area is dependent, especially since restocking of one species as fir may have trends entirely different from those of hemlock or cedar. Random sampling under these conditions can give a statistical answer as a result only of extended calculation involving indefinitely numerous records obtained at correspondingly great effort and cost.

2. "Planned sampling" as "Strip Surveys" and "Line Plot Surveys" gives statistical evidence of the degree of stocking which has occurred in the past; at the same time it gives no valid basis for prediction or for the improvement of restocking, since it does not take cognizance of causal relationships and ecological patterns.

The "Stocked Quadrat Methods" of evaluating forest reproduction is the most economical system of planned sampling since seedlings or saplings beyond the minimum limit of satisfactory stocking are placed in the category of the non-essential and thereby eliminated from the records.

3. "Selective sampling" supplies not only statistical data, but also the basis for the evaluation of the operative forces. For instance, if the effect of wind direction upon seed dispersal is to be determined, samples are taken along several radii from a centre of distribution. If the effect of topography on seedling establishment is desired, records are taken along contour lines and at right angles to the same. Temperature, light, and humidity gradients may be treated similarly.

"Selective sampling" may be approached by the method of determining the limits of conditions which pertain within an area characterized by satisfactory restocking; the maximum distance from seed trees in a given direction; the minimum and maximum soil moisture, soil temperature, air temperature, moisture, light intensity which are

tolerated by a required number of seedlings of a desirable species; the combinations of these factor limits which satisfy the range of stocking requirements. A chart of these values is essential if reproduction by seeding or planting is to be transposed from a "chance" to a "prospect." Since natural selection is now regarded as "a mechanism of generating improbability of a high order," "selective sampling" has a potential greatly in excess of other known methods.

4. In general, the investigation conducted at the University of British Columbia Forest Reserve this season has followed the principle of "selective sampling." Plots were selected within timbered areas and logged areas; within these areas plots were selected with Douglas fir, western hemlock, or cedar as the dominant form, either as trees or seedlings; in logged, burned regions, plots representing abundant, marginal, and sparse restocking of these species were selected. The ecological conditions and growth rates in each of these plots were analysed and evaluated, in so far as time and personnel permitted. The strip survey lines were selected to give a series of plots the axis of which was at right angles to the seed-producing forest front, and at the same time at right angles to the topographical contour lines. As a result of this method, certain general conclusions have been attained as enumerated elsewhere. Meanwhile, the limitation is recognized that the analysis of growth conditions and growth phenomena for a tree must extend over a period of time which will permit the occurrence of the limits of variation. If the plan followed in this early study is scientific and basic, it may be continued and amplified with complementary observations and analyses having the expectation of valuable ecological and silvicultural results.

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THE RATE OF RE-INNervation OF MUSCLE
FOLLOWING NERVE INJURIES IN MAN AS DETERMINED
BY THE ELECTROMYOGRAM*

By HERBERT H. JASPER

Presented by WILDER PENFIELD, F.R.S.C.

INTRODUCTION

THE relatively high incidence of injuries to peripheral nerves during the past war has afforded more than ample opportunity for study of the process of nerve regeneration in man. Extensive animal experimentation has also been stimulated by the urgency of the need for more precise knowledge concerning the nature of nerve regeneration and the factors determining satisfactory recovery of function following nerve lesions and operations for their repair.¹

The problem of determining the rate of re-innervation of muscle or skin following denervation is a complicated one, as recently pointed out by Young (1942), Gutmann *et al.* (1942), and Seddon *et al.* (1913). The rate of re-innervation must be distinguished from the rate of nerve regeneration since the latter is only one of the factors to be considered in the rate of re-innervation. The delay between the time of nerve suture and the return of sensation or motion may be divided into the following parts: (1) Delay at the suture line before the axon tips begin their course down the peripheral segment (7 to 10 days in animals); (2) The time required for the axon tips to grow the length of the peripheral segment into the muscle or skin (3 to 4 mm. per day in animals) as determined by histological study (Cajal, 1928) and by response to light pinching of the exposed nerve at different times following suture (Gutmann, Guttmann, Medawar, and Young, 1942); (3) Delay at the end organ before a functionally complete contact is

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established (time uncertain); (4) Time required for maturation of the regenerating nerves (increase in diameter and medullation) until the nerve is capable of conducting impulses of sufficient magnitude and frequency to produce contraction of muscle; and (5) time for central reorganization before "voluntary" impulses may be sent down the proper channels into a particular muscle—for example, voluntary movements appeared in the gastrocnemius of the cat about one month after the sciatic nerve had regenerated sufficiently to produce a muscle contraction to electrical stimulation of the nerve (23 days) in the experiments of Berry, Grundfest, and Hinsey (1944).

For purposes of this study the process of re-innervation may be considered more simply as suggested by Gutmann *et al.* (1942). There are (*a*) the initial delay at the scar, or latent, period, and (*b*) the rate of advance of functionally complete fibres capable of conducting sufficient impulses for muscle innervation. The most recent average estimate of the latter rate (*b*) following nerve suture in man was 1.5 ± 0.2 mm. per day (Seddon, Medawar, and Smith, 1943). The only satisfactory data available to these authors was for the radial nerve of which they report six cases carefully studied with rates from 0.96 to 2.42 mm. per day in individual cases. From Stopford's data on the radial nerve these authors find the average rate to be only 0.56 ± 0.03 mm. per day and are unable to explain the discrepancy. Previous careful observations, such as those of Trotter and Davies (1909), make it clear that the rate of advance of functional regeneration may be as high as 2.4 mm. per day although the average rate is usually somewhat below this figure. However, using the rate of advance of Tinel's sign in ten human subjects, Dustin (1917, quoted by Seddon *et al.*, 1943) gives approximate rates of 2 to 4.5 mm. per day for the median nerve, 1.5 to 2 mm. per day for the ulnar, and 4 to 5 mm. per day for the radial nerve. It is obvious that there is wide variability of data in man for an estimate of the rate of nerve regeneration to functional completion and that some of this variability is due to the particular criterion of recovery used in the estimates being made by different authors.

The electromyograph provides still another criterion of functional return for which data concerning rate of re-innervation are given in the present study. By this method the electrical activity of the muscle, as recorded by means of a fine needle electrode placed within it, is used as a criterion of the state of nerve supply to that muscle. Following the work of Weddell and associates, we have confirmed the observation that the spontaneous electrical activity of the denervated

muscle, as seen by means of a cathode ray oscilloscope and as heard in a loud speaker, is readily distinguished from that of a muscle with normal nerve supply, and also from that of a muscle which is in the process of recovering from previous denervation. Consequently, the electromyograph provides a sensitive indicator of re-innervation in muscle which may be used for studies of the rate of recovery following various nerve lesions in man.

MATERIALS AND METHODS

The principles used in the electromyographic analysis of the state of innervation of muscle were similar, with certain exceptions, to those established by Weddell and associates (1944). A different form of recording electrode was used in our studies consisting of a single small, very sharp steel needle coated with an inert plastic insulation except just at the point which remained uncoated owing to surface tension. The diameter of actual electrical contact with the muscle fibres into which the needle was inserted was usually about 50 to 60 micra. This monopolar exploratory electrode was connected through a condenser to one grid of a balanced high gain push-pull vacuum tube amplifier. The other grid of this amplifier was connected to a reference electrode 8 mm. in diameter placed upon the surface of the skin as near as possible to the point of insertion of the needle. A larger skin surface grounded electrode (3×6 cm.) was placed on the limb at a distance from the grid leads to minimize extraneous electrical artifacts (Plate I and Fig. 1).

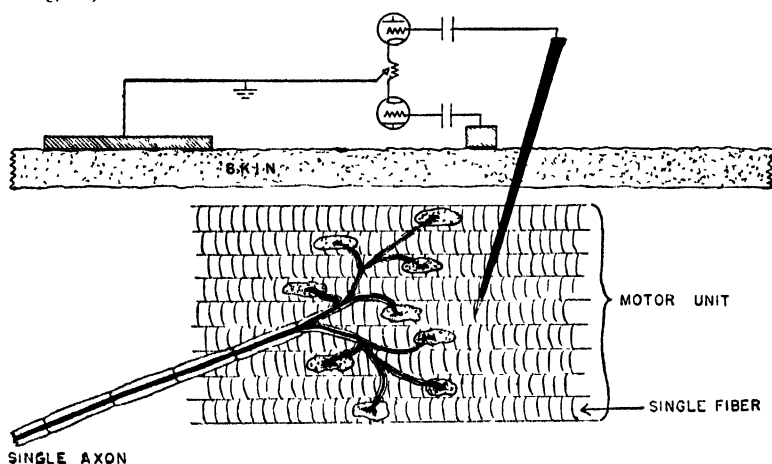


FIGURE 1.—Schematic diagram of the innervation of skeletal muscle and the method used for recording its electrical activity by the unipolar needle technique.

The spontaneous electrical activity of the muscle or that resulting from voluntary contraction was then amplified, and connected to the vertical plates of an especially designed cathode ray oscilloscope and to a loud speaker (Fig. 1). Both visual and auditory methods were used in the analysis of a given muscle and photographic record of sample "sweeps" of the oscilloscope were taken in most cases.

The unipolar needle electrode, as used in these studies, revealed the local activity from a very small area about the point of the needle as sharp and loud, while the activity from distant muscles was perceived as a distant rumble, analogous to the sharp crack of nearby lightning and the distant rumble of thunder. A large portion of a given muscle was sampled in each case by passing the needle through, and often by placing other needles in different portions of the muscle, particularly at the estimated site of entry of the nerve.

The length of the regenerating segment of nerve was approximated from measurements on the skin surface from the site of injury to the site of entry of the nerve into the muscle. These measurements cannot be highly accurate and there may be a small systematic error making all measurements somewhat smaller than they should be since no allowance was made for bending of the nerve endings as they ramify into the muscle. The percentage error cannot be very great, however, especially in the longer nerves. This simple type of measurement has the advantage of being that usually considered in clinical examinations of human subjects.

The rate of re-innervation was determined simply by dividing the length of nerve from the site of lesion to the muscle by the number of days since injury, or operation, subtracting ten days for delay at the scar.

The results of E.M.G. examinations and clinical findings in 119 patients with peripheral nerve injuries were selected for this study, from a group of over 600 cases examined. Selection was based upon the presence of satisfactory E.M.G. evidence of early re-innervation following a complete and uncomplicated nerve injury. There were 82 cases following end-to-end suture and 37 cases recovering without suture, probably axonotmesis. The distribution of nerves was as follows: ulnar 49, radial 29, peroneal 21, medial 16, and posterior tibial 4. (A few sciatic nerve injuries were included in the peroneal and posterior tibial groups.)

RESULTS

With this fine-pointed needle exploratory electrode within the substance of the muscle, the action potential of a single muscle fibre,

as was observed only in denervated muscle during spontaneous fibrillation, appeared as a negative monophasic, or slightly diphasic, wave of 1 to 1.5 milliseconds duration (Fig. 2). Its amplitude was usually about 50 to 100 microvolts. It produced a sharp crackling sound in the loud speaker.

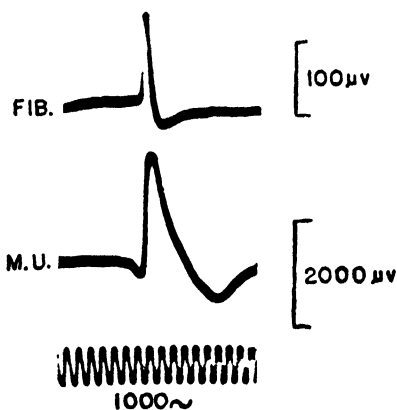


FIGURE 2.—Action potential from a single muscle fibre (above) as compared to that from a motor unit (below) which is composed of 100 (more or less) fibres “fired” by a single nerve impulse.

The motor unit action potential occurring in the normal muscle in response to a single nerve impulse from a single axon appeared as a large more or less triphasic wave of 5 to 8 milliseconds duration (Fig. 2). The larger negative phase of this wave was usually about 1 to 6 millivolts in amplitude and produced a loud “knocking” sound in the loud speaker. During re-innervation (and in certain degenerative neuromuscular diseases) the synchronization of the many separate fibres composing a motor unit is impaired and extremely complex prolonged polyphasic motor unit discharges were seen which produced a characteristic “rough” sound in the loud speaker. The one exception to this rule was the facial muscles which normally gave rise to complex polyphasic motor unit responses.

Experimental nerve lesions in animals and the study of verified nerve lesions in man have served to provide electromyographic signs of a denervated muscle which serve to differentiate it clearly from a muscle with its normal nerve supply. Within two to three weeks following a nerve lesion the affected muscles begin to fibrillate spon-

taneously and continue fibrillating as long as any healthy contractile tissue remains in the permanently denervated muscle. Fibrillation has been found to cease only when the muscle becomes cold and ischemic, when it is atrophied, or when it becomes re-innervated.

Electromyographic evidence of re-innervation was divided into three categories for purposes of the present study: (1) cessation of fibrillation or electrical silence, (2) the initial appearance of small motor units, and (3) the appearance of larger motor units compatible with the presence of visible voluntary movement.

1. *Electrical silence*

The first sign of re-innervation appeared in the E.M.G. as a decrease or cessation of denervation fibrillation (see also Weddell, Feinstein, and Pattle, 1944). Fibrillation was increased in the denervated muscle by the administration of small amounts of prostigmine (0.1 to 0.15 mg.). Curiously it has been recently found that fibrillation was decreased or eliminated by the same amount of prostigmine in a muscle which is in the early stages of re-innervation. Consequently prostigmine is a valuable aid to the differential interpretation of electrical silence, the first sign of re-innervation, but also produced by other causes.

Complete silence, without portions of the muscle showing some residual fibrillation and other portions showing early motor unit activity, was not always observed. It is thought that this is due to differences in the rate of re-innervation in different portions of the same muscle.

The fact that complete electrical silence was found in some muscles several weeks before the appearance of motor units shows that the regenerating nerve may arrest fibrillation in the muscle before it has matured sufficiently to conduct the impulses necessary for its excitation to even a slight visible contraction. In fact the effect of nerve supply on fibrillation and denervation atrophy is not due to its function as a transmitter of nerve impulses, as is shown also by the fact that complete nerve block by pressure or ischemia or deafferentation of the peripheral motor neurone does not cause fibrillation in the muscle nor does denervation atrophy occur (Tower, 1937, and Denny-Brown and Brenner, 1944).

The rates at which regenerating nerves were found to cause a cessation of fibrillation in their muscles of supply are shown in the following table (I).

TABLE I

E.M.G. SILENCE
RE-INNervation RATE (MM. PER DAY—10)

Nerve	No. Cases	Range	Standard deviation	Mean	P.E. Mean
Ulnar,	14	1.5-6.0	1.2	3.0	0.22
Radial	10	1.3-5.2	1.2	3.4	0.25
Median,	3	1.4-5.0	1.6	2.8	0.65
Peroneal	6	1.2-4.8	1.1	3.0	0.30
Post tibial,	1			2.4	
<hr/>					
All nerves	34	1.2-6.0	1.2	Mean 3.1 median 3.0	0.14

It is clear from the above table that there is a rather wide range of rates for the same nerve in different individuals, although a similar range of rates exists for each nerve. The average rate of about 3.0 mm. per day is not significantly different for ulnar, median, and peroneal nerves but the radial nerve shows a somewhat higher rate, 3.4 ± 0.25 mm. per day. This is in accord with general clinical experience that the radial nerve shows a somewhat more rapid rate of regeneration than do other nerves. It is of interest that this rate corresponds almost exactly with the average rate for the growth of axon tips in the rabbit, 3.45 ± 0.16 mm. per day, determined by the "pinch reflex" on the exposed nerve in the experiments of Gutmann *et al.* (1942). The average rate for all nerves, from our data, 3.1 ± 0.14 mm. per day, is only slightly below the rate of advance of the axon tip as determined by careful animal experiments. The median rate of 3.0 mm. per day for silence in the E.M.G. is probably the best estimate for use in clinical work. It must be kept in mind that more rapid rates, between 5-6 mm. per day, may be found in exceptional cases.

2. Small polyphasic motor units, less than 500 microvolts

The first electromyographic indication that the regenerating nerve was capable of conducting impulses was the appearance of small complex motor units, usually in response to attempted voluntary movement of the muscle examined, or of its antagonist. These first appeared as a repeated grouping of a number of fibre potentials into a complex unit of amplitude no greater than the fibrillation potentials. They then became more complex as more and more fibres were brought

into the unit and the voltage increased. Still, with amplitudes not more than 500 microvolts, as recorded with the monopolar needle electrode, perceptible voluntary movement was rarely present.

The rates for the appearance of small motor units are presented in the following table (II).

TABLE II
SMALL MOTOR UNITS (LESS THAN 500 MICROVOLTS)
RE-INNervation RATE (MM. PER DAY $\times 10$)

Nerve	No. Cases	Range	Standard deviation	Mean	P.E. Mean
Ulnar . . .	21	1.3-1.7	0.9	2.7	0.09
Radial. .	12	1.0-5.0	1.6	2.6	0.31
Median .	8	0.8-4.2	1.15	2.2	0.28
Peroneal .	11	0.6-3.4	0.9	1.6	0.17
Post tibial	2	2.0-2.9		2.4	
<hr/>					
All nerves .	54	0.6-5.0	1.1	Mean 2.4 median 2.3	0.10
<hr/>					
All nerves with- out peroneal	43	0.8-5.0	1.1	Mean 2.56	0.11

It is apparent that the rate of re-innervation to the stage of small motor units is somewhat slower than that for cessation of fibrillation, though there is considerable overlapping of ranges of rates, both being rather large. It is of interest that the difference in average rate, for small motor units, as compared to "silence," is greatest with the peroneal nerve (40 per cent less) and least with the ulnar nerve (10 per cent). The slower rate of recovery commonly observed clinically in peroneal nerve lesions may be due to slower maturation and not to a slower rate of growth of axon tips since this nerve did not differ significantly from the other nerves in rate for cessation of fibrillation.

It is felt, therefore, that the peroneal nerve should be omitted from calculations of the average rate for the appearance of small motor units for all nerves. This gives a rate of 2.56 ± 0.11 mm. per day for nerves other than the peroneal. A separate rate of about 1.5 mm. per day should be used in estimations of when to expect small motor units in the peroneal nerve.

3. *Large motor units, greater than 500 microvolts*

When motor units reached 500 to 1,000 microvolts, and were well sustained, voluntary movement became visible in some cases. The form of these units was still very complex in most instances but in some they were beginning to simplify in the direction of the normal form. Average rates for the development of the larger units are given in the following table (III):

TABLE III
LARGE MOTOR UNITS (GREATER THAN 500 MICROVOLTS)
RE-INNervation RATE (MM. PER DAY—10)

Nerve	No. Cases	Range	Standard deviation	Mean	P.E. Mean
Ulnar	14	0.7-3.5	0.7	1.5	0.12
Radial	7	0.8-2.1	0.4	1.3	0.10
Median	5	1.1-6.0	1.6	3.1	0.48
Peroneal	4	0.8-2.5	0.7	1.3	0.21
Post tibial	1			3.3	
All nerves	31	0.7-6.0	0.7	Mean 1.8 median 1.5	0.08

The most reliable figures in the above table are those for the ulnar nerve with fourteen cases, with an average rate of 1.5 ± 0.12 mm. per day. The range of values is again large, between 0.7 and 6.0 mm. per day, although this latter figure represents only one isolated case, out of the range for other cases which do not exceed a maximum of 3.5 mm. per day. The median rate of 1.5 mm. per day may be the most accurate estimate and compares well with the average estimate given by Seddon, Medawar, and Smith for the rate of recovery of voluntary motion in man (1.5 ± 0.2 mm. per day).

4. *Relations with length of regenerating segment*

In the attempt to discover why there was so much variation in rate of re-innervation in different patients correlations were made between rate, in mm. per day — 10, and length of regenerating segment. These correlations for "silence," small and large motor units are given in Table IV.

TABLE IV

RELATION BETWEEN RATE OF RE-INNervation
AND LENGTH OF REGENERATING NERVE

Silence $r = 0.23 \pm 0.10$

Small m.u. . . $r = 0.15 \pm 0.09$

Large m.u. . $r = 0.29 \pm 0.11$

None of the above correlations may be considered of statistical significance. It is curious that they are all positive, indicating a slight relationship between a longer length of regenerating distance and a higher rate of regeneration. This is the opposite of what might be expected if there is a decline in rate as the axon tips get further along down the nerve, as suggested by Seddon, Medawar, and Smith.

The explanation of this unexpected result is probably in that, with a longer length of nerve to regenerate, the lesions are more proximal and thereby nearer the anterior horn cell. This may cause a more rapid regeneration rate, at least in the initial stages, which counteracts the tendency for a falling-off in rate as the axon tips get further along in the distal segment.

CONCLUSIONS

1. The average rate of re-innervation of human striate muscle following complete nerve lesions and suture or regeneration without suture as determined by the cessation of fibrillation in the electromyogram was 3.1 ± 0.14 mm. per day $- 10$. This rate in the radial nerve was found to be significantly higher, 3.4 ± 0.25 mm. per day $- 10$. Rates differed between 1.2 and 6.0 mm. per day $- 10$ in different individuals.

2. The average rate for the appearance of small polyphasic motor units in the electromyogram was 2.6 ± 0.11 mm. per day for all nerves examined excepting the peroneal nerve in which the average rate was 1.6 ± 0.17 mm. per day $- 10$. Rates differed in individuals between 0.8 and 5.0 mm. per day $- 10$.

3. The average rate for large motor units, above 500 microvolts and compatible with visible voluntary movement, was 1.8 ± 0.08 mm. per day $- 10$. The median rate for this degree of re-innervation was 1.5 mm. per day $- 10$. The range of rates in different individuals was 0.7 to 3.5 mm. per day $- 10$.

4. The average rate of re-innervation was not decreased the longer the regenerating distance. In fact there was a slight tendency for the rates to be more rapid when the regenerating distance was longer, possibly owing to the lesion being nearer the nerve cell body.

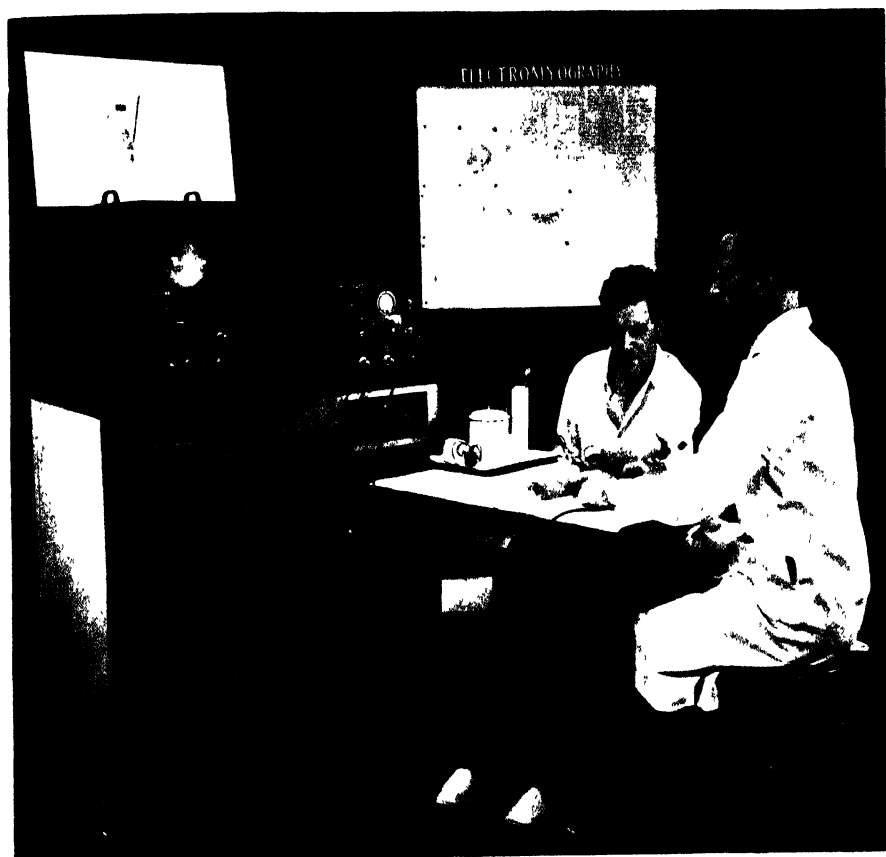
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EXPLANATION OF PLATE

PLATE I.—The R.C.A.M.C. Electromyograph Mark 111, showing cathode ray oscilloscope amplifiers, and loud speaker cabinet. The method of applying the unipolar needle electrode is shown in the insert.

PLATE I



RESIDUAL EPITHELIAL CELLS ON THE PULMONARY
ALVEOLAR WALLS OF MAMMALS

By CHARLES C. MACKLIN, F.R.S.C.

1. INTRODUCTION

Derivation

Residual alveolar epithelial cells arise from the entoderm of the lung diverticulum of the foregut. Although in the early embryo the cells of this diverticulum are uniform, they develop along different lines, one leading to the wide thin respiratory squames and the other to cells which retain some of the characters of columnar epithelium (Macklin, 1936b), and which are scattered as individuals or small groups amongst the squames. The squames occupy most of the area of the alveolar walls, but because they are not evident in ordinary microsections their sites have been termed "bare areas" (Macklin, 1937a).

Names

Though not admitted to exist by all workers, these residual epithelial cells have long been recognized and have been referred to as such and by other designations, as "septal cells," and "Nischenzellen." Max Clara of Leipzig (1936) has referred to them as "epicytes" and, though this term may not be ideal, it has already been used by the author (Macklin, 1937b, 1938a, 1938d) and will be given priority in this paper.

Silver lineation

The silver-wash method has been used for many decades to study the surface aspects of these cells, and in the older literature (e.g., von Ebner, 1899) we find excellent illustrations of the silver-line network so produced on the walls of the peripheral air channels and spaces. This method has unfortunately come into disfavour with certain workers because it has been misused, for when silver solution is allowed to act for too long it is certain to penetrate beneath the surface and involve the connective tissue fibrils, and there is then created a confused histological picture. Happily the result is very different in well-prepared material, which is able to yield much reliable information. The author (Macklin, 1938d) has briefly washed the air-space walls of cat and other lungs with a fresh 0.2 per cent aqueous solution of silver nitrate or citrate, flushed them immediately with

distilled water, and exposed them to direct sunlight or dilute photographic developer; and has found, in surface areal presentations, particularly in thick flattened frozen sections, a continuous network of silver lines, which he has traced, in favourable cuts, from the smooth-walled "conducting" part of the airway to the alveolar walls. The preparations were viewed preferably with a stereoscopic microscope. It was clear that the pattern of this network changed greatly as it was followed peripherally, for on the terminal bronchioles the meshes were relatively small and fairly uniform in size, whereas on the alveolar walls they were emphatically non-uniform, and marked off a few small plots, encircling the epicytes, from the relatively enormous fields representing the squames. The silver deposit was heavy around the epicytes, which stood out, when viewed *en face*, not only because of the dense encrustation of silver particles which marked the cell outlines, but also because their presenting surfaces were stippled with silver particles, so that they appeared as dense, minute, golden brown or dark brown bodies, contrasting strongly with the much lighter apparently bare areas which showed a few similar silver-impregnated particles here and there on their surfaces.

Though the lines of silver surrounding the epicytes were very well marked, those bounding the squames were comparatively delicate and might be broken up or even be absent altogether, for short intervals; but on the whole it is quite clear that the network is fundamentally a complete one, and serves to link the epicytes with the bronchial epithelium. In this view they are, indeed, but scattered outlying parts of the bronchial epithelium and, because of their lineage, are entitled to be called epithelium. They have often been so styled. For instance, von Möllendorff, the well-known German histologist, labels them "kubische Epithelzellen" (1933, p. 340).

A definite cell type

There is no doubt that the epicytes constitute a definite and distinct cell type, and as such are worthy of the most careful and thorough study, even though much research energy has already been expended upon them. In spite of abundant and, to the author, convincing evidence to the contrary, there are still some writers (e.g. Neubuerger, 1941, p. 561) who doubt the presence of epithelial cells upon the mature alveolar walls. The author feels that these cells are not as well known to pathologists as they should be, probably because of the unfavourable nature, for their revelation, of the microsections they study. That they must be of great importance there is no doubt. For

instance, their total volume in the human lung has recently been estimated by von Hayek (1942) to be 150 cubic centimeters. To him these cells, which he calls "alveolar epithelial cells," if all merged together, would make an "alveolar epithelial organ" the size of the spleen. Von Hayek is reported to have represented these cells as filling some of the spaces of the lung capillary network, and as sending small thread-like processes over the adjoining alveolar surfaces; and he has postulated functions for this diffuse "organ," such as fat storage, fat metabolism, and heat control. The author (Macklin, 1938d) has averred that they might become malignant and give rise to a form of primary cancer of the lung. It seemed worth while, therefore, to make a survey of epicytes as they are displayed in the microsections of ten ordinary mammalian lungs, including those of man.

Material

Most of the sections studied in this survey were cut from paraffin blocks of lung material which had been fixed by perfusion of the pulmonary blood vessels with Bouin's solution (Masson, 1929) while the chest wall was still intact and the lungs accordingly uncollapsed. There are material advantages in this method of preservation. The epicytes are shown in a morphological state akin to that of nature and free from the distortions which involve them in material fixed by bronchial filling or immersion. Slides from lungs prepared in the last-mentioned ways were also studied, usually for comparison. Many frozen and a few celloidin sections were inspected. The usual thickness was 10μ , but many sections were at 25μ , 50μ or even more, and not a few were at 5μ , or even less. The thicker sections were useful in giving *en face* views of epicytes in their position on the alveolar wall, while the thin sections showed these cells best in profile views. The majority of sections had been stained with Ehrlich's hematoxylin and eosin, but a goodly number were coloured with one of Masson's (1929) trichrome stains, such as haemalum, saffron, and erythrosin, or iron hematoxylin, light green and ponceau acid fuchsin, and some with the Mallory azan mixture or the simple crystal violet. Some special slides were also available, such as those prepared by the silver impregnation method of Bielschowsky. Most of the material was from the collection used in the study of terminal venules (Macklin, 1945).

This survey was carried through an entire series of ten mammalian types as follows: man, domestic cat, rabbit, monkey, guinea-pig, goat, dog, baboon, rat, and mouse. For each lung, many sections were looked at critically with various powers of the microscope, par-

ticularly that of the oil immersion lens, and over a hundred photomicrographs were taken to aid in a comparison of the optical images of the series. In this way a comprehensive conception of the epicyte as a major cell type of the mammalian lung was built up. First, the general findings will be given.

2. THE SURVEY: GENERAL FINDINGS

Optical presentations

At the outset it should be said that the optical picture of the epicyte presented in the sections is an extremely variable one, this variability depending on a number of factors. The cell is definitely polarized, and would seem to be set in the wall so that its main axis is, in general, at right angles to the plane of the adjacent part of the wall. That means that the histological appearance depends on the direction of section through the cell with respect to the main axis, or, if the entire cell is contained within the section examined, then it depends on the direction of the main axis with respect to the plane of the section.

Obscuration by adjoining cellular parts

Unless the section is very thin there is apt to be an infringement upon the optical field of the epicyte under examination by parts of neighbouring cells belonging to capillary walls, blood cells within capillaries, or by fibrils of white or yellow connective tissue. Speaking of optical presentations of epicytes in general, it is comparatively rare to find one which is completely free from encroachment of contiguous cellular and intercellular parts. This encroachment into the field of the epicyte is much greater in material fixed by immersion than in that fixed by vascular perfusion or bronchial filling.

Influence of fixation method

In material fixed by immersion the alveolar walls are contracted and thickened; accordingly the epicytes are squeezed out of their usual shape, and they also come to be overlapped by neighbouring cells. Thus the epicytes are not easily found in such sections. As this is the method used by pathologists it is not to be wondered at that these men are but imperfectly aware of epicytes and their attributes.

If the material has been fixed by bronchial filling the alveolar walls are made thin by pressure of the fluid within the alveolar spaces, and

this has the effect of shortening and widening the epicytes with respect to their long axes. Since the wall is stretched, the contiguous cells and parts tend to be pulled away from the epicytes so that they may be better seen.

In vascularly perfused material the shape of the cell is probably best preserved. The capillary outlines, however, often bulge out beyond the contours of the epicytes so that they appear more deeply set into the wall than they do in the bronchially filled cases.

Post-mortem change

As almost universally happens with human material, there is a delay of many hours between death and fixation, and in this time important changes occur. It may be that slight actual movements of the cells or parts thereof occur soon after death. Cytolytic changes follow the cessation of the circulation. It is obvious that the sections which the pathologist usually studies are not at all favourable for the display of epicytes, as they are fixed by the most unfavourable method after they have undergone serious post-mortem changes. In spite of these disadvantages, profitable observations may be made upon post-mortem human lung material if the observer knows what to look for and has the patience and perseverance to make the most of his opportunities.

Disease

In human material the lungs are frequently in a more or less serious state of disease, and the picture of the epicytes is accordingly altered. Parts of the lung may be devoid of them, and the tissue be so changed by pathological processes that the normal formations are completely obliterated.

Physiological state

It has been known for a long time that the epicytes can act as phagocytes, and when they do so their cytoplasm undergoes a certain efflorescence, growing in volume and becoming foam-like in structure. This similarity has led to their being called "foam cells" when in this condition. Even in the early stages of this metamorphosis they may show fine particles of dust, and apparently have the capacity to incorporate such material into their substance. As the cell grows in volume it projects farther and farther into the air space, and ultimately becomes free of attachment to the wall. Apparently it becomes amoeboid and can then make its way around upon the alveolar wall.

It is evident that such a cell is vastly different in morphology and size from the usual resting epicyte, and, indeed, can no longer be known strictly as an epicyte, but rather as an "epithelial phagocyte" or "alveolar phagocyte."

Age

It is not known what changes, if any, are shown by the epicyte which are due to age. It is certain, though, that epicytes are found in the aged, for they occurred in this series in the lungs of a man aged eighty-two years. In a child in my series they were found as early as two years and five months, and the cells then appeared to be essentially of the same character as they were in old age.

Shape

The shape of the cell as seen in the section varies with its pose with reference to the line of vision. In profile, for instance, the cell looks very different from its appearance when regarded directly from above. If thin sections cut through the cell are being inspected we may take as basic contours: (1) a cut down the main axis of the cell, and (2) a cut across the large end, hereinafter to be spoken of as the head end. When one considers the infinite variety of planes along which the cell, or the alveolar wall in which it is situated, may be cut, it is realized why the optical picture of the epicyte is such a pleomorphic one.

Then, too, as we have said, the mode of fixation has a great influence on the final form, as have also the physiological state and the presence of post-mortem change. The form of the epicyte is probably most faithfully represented in material well fixed by the method of vascular perfusion. In sections from such tissue it is possible to divide the presenting contours of epicytes into two general classes: (1) profiles, and (2) face views. However, it must be promptly admitted that each of these classes shows a great variety of form.

Profile presentations

In obtaining photomicrographs of profile views of epicytes in partitional (Macklin, 1944) alveolar walls, the ideal was striven for of selecting cells with both free, air-exposed ends in the optical field. When this is done the characteristic form is that of a carafe; or perhaps an even better form analogy would be that of a rivet with rounded head, and end of shaft slightly spread. On either side lie swollen capillaries. Fig. 1 shows such a cell cut along or near the

main axis. The large head end contains the nucleus. The surface of the head is convex in the section, so that, *in toto*, it is dome-shaped.

The protoplasm of the head shows a zone of clear spaces which are definitely chromophobe toward the stains of the ordinary mixtures such as hematoxylin and eosin. Between these clearer regions, which we may refer to as vacuoloid spaces, there are lines (really layers or partitions) of cytoplasm, which are typically eosinophilic. There is a stratum of this cytoplasm under the cell membrane. Eosinophilic protoplasm is found in the narrower neck-like part of the "carafe," which we may term the stalk, shaft, or shank.

In preparations which have been silver-washed there appear small and numerous particles of golden brown silver-bearing material adhering to the convex surface; and these particles are particularly densely concentrated at the peripheral margin of the head at the spot which, in the sections, marks the separation of the head from the neighbouring tissue of the wall. In the cat lung the previous observation (Macklin, 1938d) was confirmed that the narrow end of the stalk, presenting to its alveolar air space, is often seen to be encircled by a tiny ring of silver deposit. Not infrequently this ring is connected with the larger and denser one encircling the broad end of the epicyte by a line of silver.

This carafe shape is met with in sections relatively infrequently as compared with shapes of less regular nature arising from oblique cuts through the cell itself, or, in thicker sections, through the adjoining wall, which determine the inclination of the long axis of the cell at an angle with reference to the plane of the section. Hence we often find cells shaped like a bread-roll, without a stalk, and looking as though they were actually lying on the surface of the wall instead of being inserted into the wall like a peg (Macklin, 1938d), as is the characteristic picture of properly fixed cells so posed that the main axis is parallel with the plane of the section. It is felt, however, that this carafe or rivet shape is the characteristic one for epicytes seen in frank profile presentations.

Face presentations

In face view, Fig. 2, the epicyte is typically rounded in outline, but it may be oval, or the contour may show blunt projections. The nucleus in such views is round or oval, with a well-pencilled nuclear membrane and scattered grains of chromatin. Mitoses were not seen, but were not searched for particularly. The cell membrane is often well marked; but may appear faint or, in some places, indistinct, particularly in poorly preserved human material. Around the nucleus

is a clearer zone which may be resolved into vacuoloid spaces in well-fixed material, but which otherwise may not be clearly seen. The typically eosinophilic protoplasm extends from the marginal zone into the interior between the spaces, which present a high degree of variation in different cells. If one or two particles of dust are included in the protoplasm, the cell may be taken as embarking on its career as an alveolar phagocyte, but the rate at which this transition is accomplished seems to be a most variable one, depending upon the circumstances involving the cell. Such views do not present the stalk or shaft, of course.

Around the "face" capillaries are found, and these may project above the level of the border of the epicyte head. In silver-wash preparations, such views give the best idea of the dense silver ring which circumscribes the face of the epicyte, and also of the scattering of silver particles which are prominent over the dome-shaped presenting surface. Encroachment of bordering tissue, particularly in immersion-fixed material, has been mentioned.

In face views there is, too, a wide variety of picture depending on the relation of the plane of the face to that of the section containing it. It is the frank view that has just been described; of course, oblique face views, that is, poses in which the central axis is tipped more or less away from the plane of the section, are more commonly seen. However, unless such obliquity is marked it is often difficult for the observer to be aware of it on casual inspection of the cell.

Size

But little can be said here as to the size of epicytes, for this has been determined in only a few cases. In these the diameter of the cell in photomicrographs at 3,000 diameters magnification from man, cat, monkey, and goat was found to average about nine to ten microns. Both profile and face views were considered in this preliminary estimate, but most if not all were doubtless more or less off the ideal of frank presentation. These cells showed no particulate inclusions, so they can probably be considered as fairly representative of the typical "resting" epicytes, as contrasted with the actively phagocytic phases. It may, however, be improper to speak of any epicyte as actually resting; but this term is here used only to denote an absence of visible particulate inclusions.

3. THE SURVEY: SPECIAL FEATURES

Epicytes in ten mammalian species

Epicytes were found in every one of the many slides studied covering ten species of mammals. These species were as follows: man, cat,

rabbit, monkey, guinea-pig, goat, dog, baboon, rat, and mouse. In this list the lungs are arranged according to diminishing alveolar size as determined by Hartroft and Macklin (1943 and 1944). Samples were taken from all seven lobes or corresponding regions, of the two lungs of each animal.

Man

Epicytes were found in specimens of the following ages: 2 yrs. and 5 mos., 7 yrs., 13 yrs., 23 yrs., 31 yrs., 36 yrs., 37 yrs., 40 yrs., and 82 yrs. The histological appearance was the same at these different ages; but no special technique was used to bring out differences which are not made clear in ordinary microslides such as those used. No special determination was made to see if the numbers were different per unit area of alveolar wall at various ages; but no impression was obtained that there was any such difference. One of the outstanding findings to be emphasized here is that epicytes were constantly found in samples taken from representative ages from early childhood to old age, and the other is that these epicytes were cytologically quite comparable not only to one another, but also to those of the other nine species. The human material, as was to be expected, showed much more pathological tissue than did that of the other mammals studied, and allowance had to be made for this in making judgments as to the epicytes. Due to delay between death and fixation, the human epicytes did not stain with eosin as brightly as did those of the animals, and the vacuoloid spaces were not as sharply marked off from one another, although there were exceptions to this general statement. Since most of the specimens had been fixed by immersion, the epicytes were crowded by the surrounding tissues and it was difficult if not impossible to get anything but distorted specimens, obscured by the encroachment of the contracted and thickened walls.

Cat

The epicytes were studied intensively in three specimens, representing the bronchial filling and vascular perfusion fixation techniques. In sections fixed by the last method, excellent examples of carafe-like epicytes set into the partitional alveolar walls like plugs, and bordered by distended capillaries, were found. Fig. 1 shows a good specimen. The usual views, however, were of slanting cuts, and it was common to find the thickened head part of the epicyte without a trace of the shank, or, it might be, with only part of it. The silver-wash material (Macklin, 1938d) was re-examined, and previous conclusions were sustained.

Rabbit

Epicytes were common in the walls. The impression was gained that when epicytes become differentiated into alveolar phagocytes the protoplasm of the cell is less eosinophilic.

Monkey

Characteristic profile and face views of epicytes were found in a vascular perfusion fixed specimen. The profile presentations showed typical rivet-like forms. One such form was found in which the shank showed what appeared to be a column of the same sort of vacuoloid spaces as those typical of the head part. No other case of this kind has been observed in epicytes of any animal. On either side of the shank were capillaries, which appeared swollen and thin-walled from the perfusion technique; and it may be that the shank is made longer and narrower by the pressure of the fixing fluid in these contiguous vessels. The impression was gained that epicytes are fewer in the monkey than in most other animals, and this may be because its habitat is comparatively free from dust.

Guinea-pig

It was found that perfusion fixation was less favourably carried out in this animal than in most others, doubtless on account of the peculiar muscle formation in the pulmonary artery of this animal. It was common to find alveolar walls which had been thinned by accumulation of fixing fluid in the adjoining alveolar spaces, this fluid having apparently leaked out of the blood vessels during the perfusion. Thus the epicytes found were lower and broader than they would have been had the alveolar walls not been stretched. Epicytes with pale halos around the nucleus were common. Alveolar phagocytes, lying free in the air spaces, with a few dust grains, showed a relatively ragged and apparently confluent set of vacuoloid spaces.

Goat

Epicytes in face and profile presentations were commonly found in the two specimens examined, fixed by vascular perfusion. The direct sequence of bronchiolar into epicytic epithelium was frequently demonstrated.

Dog

Epicytes were numerous in their various positional aspects. In looking over a section the speculation was excited of what it is that

causes the efflorescence into the phagocytic stage. This potency seems to have been developed in the peripheral members of this entodermal system—that is, in the scattered epicytes of the alveolar walls; and the temptation to associate it with the appearance of the respiratory squames is strong, for with the squames came the need for their protection from incoming particles of dust, and the alveolar phagocytes seem to have been elaborated for this specific role.

Baboon

Good views of “resting” epicytes, that is, those which have not yet started to “rise” like a loaf of bread, in their transition into alveolar phagocytes, were afforded. Dust-containing transitional forms were also seen.

Rat

The impression was left that dust-containing alveolar phagocytes in this animal were more common than in a number of others, such as the monkey. This may be because the rat lives habitually in a dusty environment. Face and profile presentations of epicytes were repeatedly observed. Cells with foamy protoplasm extending out in a tongue-like manner were seen, and it may be that such extensions represent the “flanges” (Bremer, 1938) or “thread-like processes” (von Hayek, 1942) described in the literature.

Mouse

This animal had the smallest alveoli encountered in the series of Hartroft and Macklin (1943 and 1944). Quiescent epicytes, and particularly phagocytes arising from them, seem therefore large in comparison with the neighbouring air chambers. Foamy alveolar phagocytes were numerous. Since the respiratory bronchioles of this animal are very short (some workers might even consider them to be absent), it is common to find places in the sections where the bronchiolar cuboidal epithelium may be traced over into the thinner alveolar walls where epicytes, resembling the bronchiolar epithelium, are found. From this analogy of structure, and from this histological continuity, the conviction that the epicytes are derivatives of the primitive bronchiolar epithelial cells is strongly formed.

Summary of the ten mammal series

Since epicytes are universally found in the lungs of these ten mammals it would seem reasonable to regard them as fundamental

lung cells. Therefore it would hardly be expected that mammalian forms would be found which did not show epicytes. We are impressed with their ubiquity throughout the lungs, for all parts thereof would seem to be equipped with them. In most of the lungs studied there were representatives of all seven lobes, and in the human lungs the tissue corresponding in location to that of the seven lobes was sampled. The conclusion was unavoidable that epicytes are found throughout the normal mammalian lung substance in uniform distribution, for the lobes. That they are more common on certain marginal alveolar bases, as those of the pleura, and perivascular, peribronchial or septal tissue, than on partitional ones, has already been noted (Macklin, 1938d). In these locations they have only one air-exposed face.

4. DISCUSSION

A fundamental cell type

The epicytes, like the bronchial epithelium, the fibrocytes, or any other well-differentiated cell type, look the same and react similarly regardless of whether they are in a cat or a man or any other mammal. Thus these cells are infinitely broader in their scope than animal species. They are common to many species. In this study no species differentiation in morphology or function was noted, though this point was not specially covered.

Combined mass

With such a universality of distribution of epicytes not only in any given mammalian lung but throughout the mammalian series, it would seem that this cell is important. It may even be regarded, collectively, as a vital organ. Sjöstrand and Sjöstrand (1938) say they amount to about 10 per cent of the lung tissue. Von Hayek's (1942) estimate that the combined mass of the epicytes in the human lungs would constitute an organ the size of the spleen is impressive; and even if it should turn out to be an exaggeration we may still be sure that the combined mass would be a considerable one.

Phagocytic potencies

The ability of the epicyte to assume the function of phagocytosis, defined as the phenomenon of incorporating particulate matter, such as dust particles, into the cytoplasm, is widely recognized. Bratianu and Guerriero (1930) discuss this, and give more than 160 references to literature relating to this cell. With this function these authors link the allied phenomenon of colloidopexia.

Cytoplasmic increase. The protoplasm between the vacuoloid spaces would seem to increase in volume, and the cell membrane obviously enlarges. With these alterations there are combined certain phenomena of motion usually referred to as amoeboid movements.

Amoeboid manifestations. The projection out from the cell of tongues or threads of protoplasm has been referred to, and this seems to be an aspect of its phagocytic and amoeboid potencies. To the author these extensions seem always associated with alveolar phagocytes rather than with quiescent epicytes. It may be that they can sweep over the bare areas and lick up any foreign particles which they may find thereon. Actual locomotive ability is acquired by the cell, which is apparently able to move over the alveolar wall and even insinuate its way through a comparatively narrow pore. To the cell may be ascribed a certain directive ability in that, when loaded with debris, it is able to make its way to the ciliated epithelium (Macklin, 1938b), "walking the tight-rope" of the devious edges of the walls of the alveolated ducts and respiratory bronchioles (Macklin and Macklin, 1942, p. 222) in the process. In the effecting of their phagocytic purpose, if so it may be characterized, they develop a certain property which may be termed a preferential adhesiveness as compared to the squames of the alveolar wall, so that the particles of dust are differentially attracted to the phagocyte and adhere to it rather than to the wall. So they would seem to be removed. That this is not a perfect mechanism, however, is obvious from the finding of so much particulate matter—mainly carbon pigment—in the alveolar wall itself and in the lymphatic system, where its presence may be regarded as "accidental" (Macklin, 1938b). Faulty as this cleaning mechanism is, it would seem essential, at least in dusty environments, to the prolonged functioning of the lung.

Conditioning of the alveolar wall

Von Hayek (1942) has mentioned other possible functions of epicytes, such as fat storage and fat metabolism. I have not studied these points. I have surmised that they may play a part in the proper conditioning of the alveolar wall. It seems certain that the outer surface of the extremely thin capillary walls must be kept always in proper condition. We are told that this outer capillary surface is covered with a fluid film (Terry, 1926), but we are in the dark as to the exact nature of this fluid film. It is so thin that it cannot be well studied histologically. It may be that the epicytes contribute something to this fluid which renders it better adapted for its part in gaseous

interchange. The finding, on the apparently bare areas in sections from silverwash material in the cat, of a few fine particles of golden brown material like those which are closely scattered over the exposed larger face of the epicytes, may perhaps indicate a causal origin, which would put the epicyte in the class of a secretory cell. It may be that it contributes something to the circumscribing fluid film of the alveolar capillary wall which is advantageous in external respiration.

Site vacaton

After an epicyte has effloresced and taken itself off, what happens to the site? It is difficult to answer that question. If the epicyte should become dislodged from its position in a partitional alveolar wall it would simply drop out like a peg from a socket, and the socket would then become an alveolar pore (Macklin, 1938d). In support of this view is the finding that pores increase with age (Macklin, 1936a). However, no evidence came to light in this series that the epicytes decrease in number with age in the human subject. It may be that when an epicyte is about to become free as a phagocyte it divides by mitosis, one part remaining *in situ* while the other is liberated; but I have not, in this series, found mitotic figures in epicytes. I do not deny that they may occur, and a later note will be made on this point. It is difficult to think of the epicytes as not being renewed, for, if they were not, then we would expect a gradual diminution of their number, but, as already stated, they have been found in this series in the alveoli of a man of eighty-two years, apparently in unlesened quantity. One would have expected that they would have been exhausted long before that extreme age if they had not been renewed. Some workers think that alveolar epithelium can be regenerated from "septal cells," but others (Herbut, 1944) look to the basal cells of the bronchioles for the source of this regeneration. I have found no evidence of any immigration of cells into the alveolar walls from nearby bronchioles.

Membrane-forming potencies

The view is held that these cells may, under abnormal stimulation, proliferate and form a continuous membrane over the alveolar wall. Such formations have been found in lungs after exposure, during life, to certain deleterious fumes, as those of nitric acid (Loeschcke, 1910), osmium tetroxide (Macklin, 1938a, 1938c), and also in certain tumor formations (Neubuerger, 1941), and it seems not unlikely that the epicytes have proliferated to create these. In acting thus they would be returning to a simulation of the embryonic condition.

Evolutional note; the single capillary net

It is of interest to visualize the epicytes in relation to the history of the alveolar walls in which they are situated. In the evolution of the partitional type of alveolar wall we have a single capillary net coming into existence (Macklin and Macklin, 1942, p. 221), which replaces the originally double one. The alveolar wall becomes much thinner and capillary loops protrude now to this side, now to that, as they weave their way across the wall. With this vascular change has come a change in the external relations of the surviving mural epithelial cells, the epicytes, which come to face two ways. We have a head and a base, or end of the shank, each contiguous with air, or at least with the film of fluid which separates the protoplasm of the cell from air. There is here the same fundamental relation of the epithelial cell to water that exists in the gills of fish, and in amphibian larvae; that is, the cell is covered, on its exposed surface, by a film of water, but with the difference that the mammalian epicyte has two such surfaces when it projects through a partitional alveolar wall, whereas the epithelial cell of the gill has but one. As already noted the epicytes found in marginal alveolar bases abutting upon the connective tissue of the pleura and other parts have but one exposed surface.

5. SUMMARY

1. Residual epithelial cells, herein called epicytes, were found in the various parts of the lungs of ten different species of mammal, viz. man, cat, rabbit, monkey, guinea-pig, goat, dog, baboon, rat, and mouse. They were scattered among the apparently bare areas of the alveolar walls.

2. Throughout this series they showed the same fundamental structure, staining reactions, and presumptive habits of life.

3. It seems probable that they are universally distributed in the lungs of normal mammals, and constitute a definite and important pulmonary cell type.

4. Preliminary determinations of their size indicate that they average, in sections, from nine to ten microns in diameter.

5. The findings indicate that collectively they compose an important, probably a vital, pulmonary organ or sub-organ.

6. They are polarized derivatives of entodermal epithelium, and are typically posed with the main axis at right angles to the plane of that part of the alveolar wall in which they are situated.

7. On alveolar walls of the partitional type, which have two air surfaces, they are set into the wall like a peg in a socket; and in silver-

washed preparations rings of silver deposit have been found encircling both of the presenting surfaces of the epicyte.

8. The shape of the epicyte is greatly different in profile and in face views. In profile it is that of a carafe or rivet, with a large dome-shaped head end containing the nucleus and a shaft or shank whose end, in partitioned alveolar walls, presents upon the opposite alveolar surface. In marginal-type alveolar bases the cells rest on connective tissue.

9. Typically, in cross-sections of alveolar walls, in material fixed by vascular perfusion, a dilated capillary is seen on either side of the shank of the epicyte, and the walls of the capillaries are then often seen to project into the air spaces beyond the level of the shank end of the epicyte, and sometimes even of the head end.

10. The face views of epicytes show usually a rounded or oval contour, which may have short blunt extensions; and also a perinuclear vacuoloid zone of variable extent. This zone may appear as a mere halo around the nucleus in improperly fixed material.

11. The protoplasm of the epicyte is eosinophilic, and shows a marginal zone with extensions therefrom into the interior, which enclose a set of vacuoloid spaces, sometimes called vacuoles, granules, or globules. These spaces may be confluent. The rounded or oval nucleus typically has a sharply marked membrane and scattered granules of chromatin.

12. The shape of the epicyte varies greatly depending on the mode of fixation used. With material fixed by vascular perfusion under moderate pressure the frank profile presentations are as synopsized in (8); but after bronchial filling the cell is much lower, due to attenuation of the alveolar wall, and appears as a relatively broad bun-shaped mass, often with little or no visible shank or stalk. The contiguous capillaries, in such a preparation, are more or less collapsed, as a rule.

Frank face views made after this fixation method show an expanded contour with withdrawal of contiguous tissue from the periphery of the cell.

If the material, as is usual in autopsy specimens, has been fixed by immersion, the epicyte shows in axial sections usually a lesser height as contrasted with the vascular perfusion cases, but a greater height as compared with the usual case after bronchial filling; and the direct profile view is altered because of the general shrinkage and thickening of the wall, causing adjoining capillaries and other wall tissue to intrude upon the optical field. Frank face views of immersion-fixed material show usually a diminished presenting area of the epicyte,

and encroachment upon the territory of the epicyte by contiguous tissue.

13. After any type of fixation, however, the optical picture seen depends greatly on the direction of the section with respect to the general plane of the alveolar wall in which the observed epicyte is placed; and as most sections are cut obliquely to the walls, rather than at right angles to them, it follows that there is a great variety of form in the epicytes presented in microsections. Most of them are cut so as not to include the shank, so that this part of the cell is comparatively unknown to histologists.

14. Because of the unfavourable nature of the material they inspect, due to post-mortem changes having occurred in it, and to its involvement in disease processes, and also because of the unsuitable mode of preparation, pathologists do not have a good opportunity to study epicytes in human material. Frequently, however, they see the epicyte derivatives, the alveolar phagocytes, which often are prominent in the sections which they routinely study.

15. The view is sustained that epicytes have within them the potency to become enlarged, apparently through the taking-in of the fluid and particulate matter of their environment. When thus metamorphosed they are known as alveolar phagocytes; and show evidence of colloidopexic as well as phagocytic properties. They also show evidence of amoeboid movement and directional inclinations toward the ciliated epithelium of the bronchial system connected with their alveoli. Their locomotion is skilful, and they can become attenuated, to crawl through small pores; and can "tread" the thin edges of partitions which compose the sieve-like walls of alveolar ducts and respiratory bronchioles, in making their way to the "escalator" of the ciliated epithelial membrane.

16. Their phagocytic potency is particularly directed to the ingestion of foreign dust particles which gain entry in the inspired air. Parts of the protoplasm become wrapped around these particles, which are gradually accumulated until the load is sufficient to warrant the retreat of the phagocyte from the field of action. This activity tends to clear the alveolar wall of such particles, but the clearing may be incomplete, due to the unfavourable nature, or excessive quantity, of the offending material.

17. In ordinary phagocytosis of particulate matter, and in colloidopexia, mobile tongue-like extensions of the phagocytes may play a part.

18. Because the epicytes are of an epithelial nature they ap-

parently retain the potency to revert to their earlier embryonic state of units in a layer or membrane when certain harmful stimuli are brought to bear on them. Such stimuli are provided by some toxic gaseous materials, as osmium tetroxide. In such cases the alveoli may become lined by a membrane simulating that of the embryo in certain ways; and this, being less favourable to gaseous diffusion than the normal alveolar wall structure, may harmfully interfere with external respiration. Epicytes thus possess in latent form a mitotic potency in high degree.

19. This mitotic potency, theoretically, may manifest itself in malignancy; and there is no evidence to disprove the supposition that primary carcinoma of the lung may originate from the epicyte, though such a transformation would be difficult to prove.

20. It may be that the epicyte has a useful role to play upon the alveolar wall in conditioning the surface fluid film; for fine particles of silver deposit have been found on the bare areas of silver-washed alveoli which resemble those on the exposed surfaces of the dome-shaped heads of the epicytes. It is thus suggested that the epicytes may have some sort of secretory function, giving rise to material which absorbs silver and appears as dark particles. Thus the epicytes may be a useful accessory in the general function of external respiration.

21. Pure "foam" cells, containing a prominent vacuoloid apparatus, but no visible particulate inclusions, are seen, and these apparently have developed from the epicytes.

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FIGURE 1.- Profile view of an epoxide (ep) in the alveolar wall of the lung of a cat. See text for description. S76-1C-1RU-10 X750.



FIGURE 2.- Face view of an epoxide (ep) of the alveolar wall of the lung of a mouse. See text for description. S73-17M-1RU-13 X750.

DEVELOPMENT OF THE LATERAL LINE ORGANS
IN SALMONIDS

By FERRIS NEAVE

.Presented by R. E. FOERSTER, F.R.S.C.

MATERIAL AND METHODS

THE chief basis of the present account has been provided by a series of specimens of hatchery-raised spring salmon (*Oncorhynchus tshawytscha*) in various stages of embryonic and post-embryonic development. The samples were preserved in formalin, the egg membrane being first removed in the case of embryonic material. Whereas previous accounts of the development of the lateral line sense organs of fishes have been based on a study of sections, preparations in the present instance were made by removal of the skin which, after staining in alum haematoxylin and dehydration with dioxane, was mounted flat.

In addition to the series of spring salmon, certain stages of cut-throat trout (*Salmo clarkii*), brown trout (*S. trutta*), and speckled trout (*Salvelinus fontinalis*), prepared in the same manner, were examined. Some sectional material of the latter two species was also available. This had been fixed in Bouin and prepared by the ordinary paraffin method.

Measurements given in the following account represent the length of preserved specimens to the end of the vertebral column. Owing to the small size and bent posture of the younger embryos these measurements are only approximate for the earlier stages. In the case of the spring salmon that were used the development recorded took place between 50 and 160 days after fertilization of the eggs. About 105 days elapsed between fertilization and hatching of the alevins. Since these periods presumably vary considerably under different conditions, the general state of advancement of the individuals has been indicated by referring them to certain recognized developmental stages. The stages defined by Pelluet (1944) have been found convenient for the present purpose and have been adopted.

DEVELOPMENT OF THE ORGANS IN THE SPRING SALMON

Sensory cord

The earliest stage examined was 50 days after fertilization, this being nearly half-way through the period spent in the embryonic condition. At this time (Stage 10) the eyes are pigmented and the

full number of body somites (usually about sixty-seven to sixty-nine) has been almost or quite attained, although the posterior ones are still very small. The length (straightened) of the preserved embryo is about 9 mm.

A sensory cord is present, extending along the lateral line from the neck region to a point level with the anus. Throughout most of its length this cord is two to four cells wide, the cells being more or less spindle-shaped with large nuclei (Fig. 1). At its posterior end, however, the cord is enlarged and consists of numerous smaller, closely packed cells. This condition presumably results from the rapid multiplication of the terminal cells of the cord and the resistance encountered from the surrounding epidermis (Fig. 2). No sense organs can be distinguished at this stage.

Primary sense organs

At 57 days (Stage 11) the cord has grown back almost or quite to the caudal fin. While most of the cells of the cord have become somewhat thinner and more elongated, others at intervals are forming small groups which can be recognized as incipient sense organs. These appear first in the anterior trunk region and each lies at or just behind the point at which a myoseptum meets the skin. The same condition is found in the posterior trunk region of more advanced embryos (Fig. 3).

The sense organs become more sharply defined through an increase in the number of their constituent cells and the orientation of these at right angles to the cord. Differentiation into sensory and supporting cells also proceeds (Figs. 4 and 5). By 79 days (Stage 12) at a length of 14.75 mm., a sense organ of this series can be recognized for each metamere of the body. Beyond these, on the caudal fin, the cord appears to fork and terminate in several organs. These terminal organs are more advanced than those immediately anterior to them.

Accessory sense organs

At about this stage (Stage 12) the development of certain sense organs outside the original series can be seen. These, in surface view, appear as more or less circular aggregations of cells lying just dorsad to certain members of the primary series. In the 79-day embryo about twelve of these organs were present, distributed rather irregularly throughout the region corresponding to the first twenty-five metameres of the trunk. In some instances they presented the appearance of buds originating from members of the primary series or from a closely adjacent part of the sensory cord (Fig. 6). Near the posterior limit of their occurrence, however, a condition was sometimes ob-

served suggestive of a backward-growing cell mass in whose wake sense organs were being laid down (Fig. 7). It is possible, therefore, that not all the accessory organs originate separately from the original sensory cord. In any case each accessory organ quickly becomes an entirely separate structure, whereas the primary sense organs remain linked together by the persisting cord (Figs. 8 and 9). By the time of hatching (Stage 13), about 105 days after fertilization, accessory organs are present at least as far back as the fortieth metamere. With the growth of the body they frequently become considerably displaced from their initial positions adjacent to the primary organs. Alevins and young fry showed about twenty-two to thirty accessory sense organs distributed along the length of the body except at the extreme posterior end. The number and position of these organs are not always the same on the two sides of the body.

Intercalary sense organs

Very shortly after hatching (Stage 13) a third set of sense organs begins to develop (111 days, 22.5 mm. in the material examined). These are formed on the sensory cord, interposed quite regularly between the members of the primary series. Appearing first as a swelling of the cord, these organs develop in the same manner as the primary organs, which they eventually resemble closely in size and form (Figs. 8, 7, and 9). The appearance of these intercalary organs takes place almost simultaneously throughout the length of the body. In the 160-day alevin (Stage 15, 29.6 mm.) the intercalary sense organs are still recognizably smaller than the primary organs but resemble the latter in form and structure.

The lateral line system of the fry can be considered as being complete at this stage. The condition persists until scale formation commences at a length of about 33 mm. (Fig. 9). Subsequent changes in the lateral line associated with scale development are not dealt with here.

COMPARISON OF SPECIES

The sequence of developments in the other species examined is the same as that described for the spring salmon, although the time intervals varied, probably in accordance with both specific differences and external conditions.

At the time of hatching, cut-throat trout (*Salmo clarkii*) showed a complete series of primary sense organs. Accessory organs were present on the anterior part of the body. No development of intercalaries had taken place. All series of organs were present when a length of 28 mm. had been attained.

Speckled trout (*Salvelinus fontinalis*) at the time of hatching (Stage 12-13) showed a less advanced condition, only the primary organs being present and these not well differentiated in the posterior region. At a length of about 20 mm. (which in this instance was not attained until more than two months after hatching) the formation of accessory sense organs appeared to be complete and development of the intercalary organs was just beginning. The latter organs showed progressive development in specimens of 23 mm., 27 mm., and 31 mm. In the last, after the formation of scale papillae was well under way, the intercalaries were still slightly smaller than the primary organs.

In the few specimens examined, the number of accessory organs varied from about seventeen to twenty-seven in the cut-throat and from eighteen to twenty-one in the speckled trout.

DISCUSSION

Wilson and Mattocks (1897) described the origin of the lateral line in the salmon (*Salmo salar*) from an anlage which also gives rise to the auditory sac and the superficial sense organs of the head. The development was traced to the twenty-fifth day, at which time the lateral line rudiment consisted of a wide anterior thickening of the ectoderm, passing posteriorly into a short, well-defined rod.

Beard (1884) had previously described the development of the sensory cord in the brown trout (*Salmo trutta*). He says that it commences in the neck region, opposite the hyoid arch, and grows back longitudinally along the whole length of the body. The sense organs develop from segmental thickenings which appear after the cord is established throughout the length of the body.

Rode (1929) gives a somewhat vague account of the developmental process in the same species. He says that at stage H of Henneguy, when the embryo is about 3 cm. long and has eighteen to twenty-two somites, the rudiment of the lateral line is seen as a thickening of the epidermis, formed by a row of cells. These cells at regular intervals fold in toward the interior of the body, forming cellular buds around which the epidermal cells congregate to form the definitive organs. These statements regarding the development and constitution of the sense organs are not in accordance with the present writer's observations on related species.

Beard's brief account is in essential agreement with the description given in the present report, up to the time of appearance of the primary sense organs. The present writer does not know of any satisfactory account of the developments beyond this stage. Both Beard and Rode were aware of a subsequent increase in the number of sense organs

(the "accessory organs" of the present account) but say nothing regarding their frequency, distribution, or development except for a foot-note by the earlier investigator in which it is stated that "the number of sense organs is increased . . . by division of the primitive segmental ones." The close juxtaposition of two organs of equal size frequently gives an appearance of such an origin, and the present writer admits to having previously held this view. As indicated earlier in this paper, he now holds that these organs are formed by a process of outgrowth or budding, somewhat similar to that which has been described in the accessory organs of *Amblystoma*, *Rana*, and *Hyla* (Stone, 1933).

In the above-mentioned amphibians the primary sense organs are laid down as disconnected clumps of cells in the wake of a lateral line primordium which migrates posteriorly along the side of the body. Wilson (1891) describes the virtual disappearance of the lateral line cord between successive sense organs in the sea bass (*Serranus atrarius*). Beard says that in the brown trout the lateral line between the organs thins out until it is quite impossible to find it in sections, although he considered it probable that a connection persisted. As already indicated, the present writer's preparations show clearly that the cord not only persists in its entirety, but that the portions between the primary organs later give rise to a very characteristic new series of organs. This remarkable intercalary type of development appears to be associated with increasing body size which is not accompanied by increasing size of the primary sense organs and hence permits a numerical increase in the sensory apparatus.

In the spring salmon the full development of the lateral sensory system appears to coincide fairly closely with the time when the fish emerges from the gravel and begins a free-living existence. It is not certain, however, that this is the case with all related species.

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EXPLANATION OF PLATE I

- FIGURE 1—Portion of sensory cord of 50-day embryo. $\times 450$
FIGURE 2—Posterior end of sensory cord of 50-day embryo. $\times 100$
FIGURE 3—Sensory cord, showing incipient primary sense organ. Tail region of 79-day embryo. $\times 450$
FIGURE 4—Primary sense organ in more advanced stage. Tail region of 79-day embryo. $\times 500$
FIGURE 5—Sensory cord and two primary sense organs. 79-day embryo. $\times 225$
FIGURE 6—Development of an accessory sense organ. 79-day embryo. $\times 450$
FIGURE 7—Apparent formation of accessory sense organ by process of budding from previously formed accessory sense organ. 130-day alevin. $\times 225$
FIGURE 8—Portion of lateral line of 111-day alevin, showing incipient intercalary sense organ. $\times 225$
FIGURE 9—Portion of lateral line of fry, 33 mm. $\times 112$

a.s.o., accessory sense organ

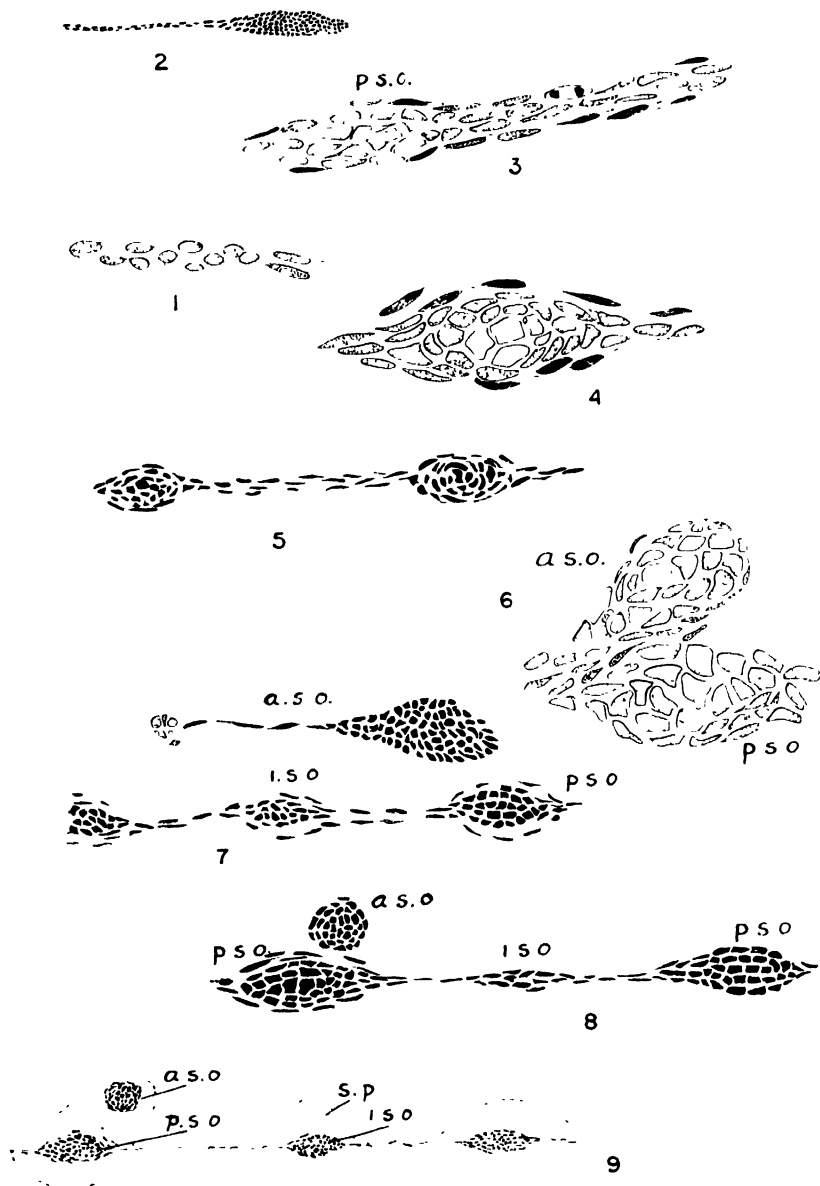
i.s.o., intercalary sense organ

p.s.o., primary sense organ

s.p., area occupied by developing scale papilla.

In all figures the anterior end is towards the left.

PLATE I



GLYCINE TOXICITY AND PYRIDOXINE REQUIREMENTS
IN THE WHITE RAT

By EDOUARD PAGÉ and R. GINGRAS

Presented by GEORGES MAHEUX, M.R.S.C.

INTRODUCTION

THE partial inhibition of growth which is caused by the addition of gelatin to a casein diet is well known (9, 10). A similar effect has been ascribed to proline, phenylalanine, and glycine (9, 10). Attempts to supplement gelatin with the lacking amino acids have generally given poor results (10, 13), while the growth response to the administration of pure amino acid mixtures has been satisfactory in some cases (11) and not in others (1, 3). Inasmuch as these various diets contained all the essential amino acids, it is clear that we are dealing primarily with an improper balance of these acids. Snell and Guirard (14) have shown that the toxicity of glycine for *Streptococcus lactis* could be counteracted within limits by pyridoxine. Fishman and Artom (7) have demonstrated the high toxicity of d1-serine when this amino acid is given by stomach tube to pyridoxine deficient rats. The toxicity is due to the unnatural form of serine (2) and causes among other things a decrease in kidney codecarboxylase (pyridoxal phosphate) (8). The effectiveness of nicotinic acid in correcting the amino acid imbalance and growth inhibition caused by the addition of gelatin to a casein chick ration has just been reported by Briggs (4). A tryptophane supplement was shown to be equally beneficial. It appears that extensive changes in the intestinal flora may account for the action of nicotinic acid (12). It thus seems that the "toxicity" of gelatin and of certain amino acids may be due to a relative lack in some particular amino acid, to changes in the intestinal flora, or to higher requirements for a specific vitamin (through competitive inhibition or otherwise).

In view of the various and sometimes undetermined levels of pyridoxine used in previous experiments, it was thought worth while to determine the effect of a high pyridoxine supplement on the toxicity of glycine in the rat.

EXPERIMENTAL PROCEDURE

Young rats were placed for one week on a diet deficient in B-vitamins.¹ The rats were then divided into three groups. The first

¹The basal ration had the following composition: purified casein (Smaco), 10; sucrose, 79; Mazola oil, 4; salts (Steenbock Salts 40), 4; Cellu-flour, 2; cod liver oil-wheat germ oil mixture, 1.

group received a full supplement of B-vitamins,² while the second group received the same supplement with the exception of pyridoxine. The third group was given a daily injection of 25 micrograms of thiamine hydrochloride. At the end of the third day of supplementation, each group was sub-divided into two, the rats being paired as to weight and as to growth response to the vitamin supplements. These were continued throughout. Ten rats were thus paired in the first group, twelve in the second, and ten in the third. To one sub-group on each type of vitamin supplements, glycine was administered

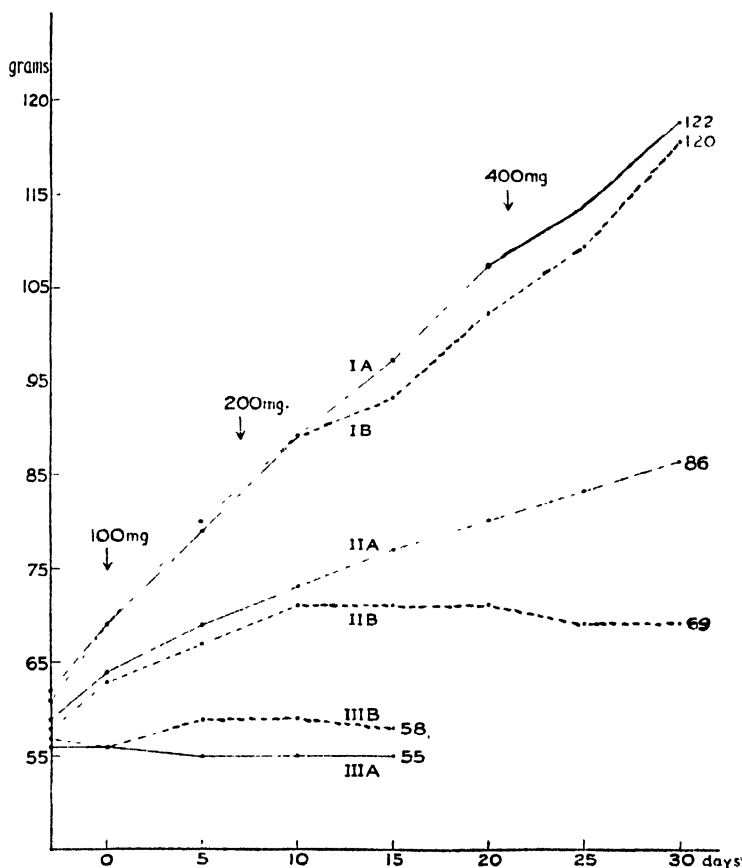


FIGURE 1.

²One hundred grams of ration contained: thiamine hydrochloride, 0.5 mg.; riboflavin, 0.5 mg.; pyridoxine hydrochloride, 1.0 mg.; calcium pantothenate, 5.0 mg.; nicotinic acid, 5.0 mg.; choline chloride, 100 mg.

by stomach tube in 2 cc. of water at the rate of 100 mg. daily during the first week, 200 mg. during the following two weeks, and 400 mg. during the balance of the experiment which lasted thirty days.

RESULTS

The growth curves are shown in Fig. 1. "O Day" marks the beginning of glycine administration, the previous portion of the growth curves showing the initial response to vitamin supplementation. Of the rats on a full vitamin supplement (group I), those receiving glycine (IB) made an average gain of 51 grams as compared to 53 grams for the controls (IA). The control group (IIA) on the ration deficient in pyridoxine gained an average of 22 grams as against 6 grams for the group receiving glycine (IIB). The latter stopped growing after ten days. Comparison of body weights in the third group had to be abandoned by the end of fifteen days due to mortalities. It was noted, however, that four of the five rats receiving glycine showed definite signs of acrodynia, while these symptoms were absent in the control rats.

DISCUSSION

On a diet well supplemented with pyridoxine (1.0 mg. per 100 grams), and under the conditions of the experiments, the toxicity of glycine could not be demonstrated conclusively. The significance of the temporary lag in growth of the experimental group (IB) is questionable and moreover, this lag was overcome at a time when the rats were receiving massive doses of glycine (400 mg. daily, or nearly 4 grams per kilogram of body weight). The arrested growth of the pyridoxine deficient animals receiving glycine (IIB) is in marked contrast to the smooth growth rate of similarly deficient rats not receiving glycine (IIA) and to that of rats receiving both pyridoxine and glycine (IB).

It is evident that some relationship exists between pyridoxine and glycine metabolism and it is further apparent that the reported toxicity of glycine can be alleviated to a large degree if not entirely by a high intake of pyridoxine. It is recalled that pyridoxine requirements for growth have been determined with rations containing casein, a protein of high biological value. There is already some evidence that the protein level of the ration may influence these requirements (5, 6). In view of the role of pyridoxal phosphate in transamination and decarboxylation reactions, it is not unlikely that the biological value of the proteins fed may also have some effect on

the requirements for pyridoxine. If this were the case, the present minimum standard of 10 micrograms daily would be inappropriate where a pronounced imbalance of amino acids exists. Work is in progress on that point.

SUMMARY

The administration of massive doses of glycine by stomach tube (100 to 400 mg. daily) has little or no effect on the growth of rats receiving a high supplement of pyridoxine. Complete growth inhibition occurs in the case of rats on a pyridoxine-free diet.

It is suggested that amino acid imbalance in the diet may increase the requirement for pyridoxine.

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EXPERIMENTS IN BIRD MIGRATION

By WILLIAM ROWAN, F.R.S.C.

I AM afraid I feel very self-conscious in delivering this invitation paper to Section V. As the recipient of the Flavelle Medal, I can only thank you for the honour you have bestowed on me and express a regret that I should feel myself unable to concur in your generous decision.

My first impressions of the migratory movements of birds on a large scale were obtained when I was convalescing at the biological station at Blakeney on the Norfolk coast of England towards the close of the last war. The sight impressed me so deeply that I decided, there and then, with the innocent optimism of youth, to attempt an experimental analysis of some of its factors. What this decision led to is the topic of this address.

The first step was to learn something of birds and their ways, and the immediately ensuing years saw me sedulously plying the trade of ornithologist, at first in England and thereafter back in Canada. On perusing the literature, it was apparent that the subject had generally been treated in a one-sided manner, either by bird men who knew little of academic biology, or more occasionally by trained biologists who knew nothing about birds. The obvious first step seemed to be to learn something about both sides of the question and at least have all its aspects available for consideration. Field investigations soon became revealing and suggestive. Edmonton, being as far north as it is, and on a migratory highway, proved an excellent spot for observation. The most striking single fact that emerged was the incredible regularity of migratory movements as a whole, particularly of the larger species. This fact in itself actually provided the key to the subsequent experiments which were based on the assumption that regular migrations must hinge on some external stimulus, an environmental timing mechanism, itself of consistent regularity. In our northern environment only one such factor appeared to fulfil this requirement—variations in day-length.

A second fact of obvious significance that transpired through field work and the persistent collection of specimens, was the advanced state of the gonads of birds coming north through the Edmonton district. In view of the theories then current with reference to interstitial cells, sex hormones, and sex behaviour, one only had to suppose that the migratory journey was itself but a particular phase

of sexual behaviour, as much dependent on the development of the gonads as the characteristic spring antics in which most birds indulge, to establish a practical working hypothesis for an experimental start. If one could artificially stimulate the gonads to spring activity in the fall, one might thereby induce the owners, when released, to go north instead of south in the autumn.

Up to 1924, when our first attempt was staged, Loisel's belief that the spring development of the gonads in birds depended on the rising temperatures of spring was, as far as I know, universally accepted. It was apparent, however, at the Edmonton latitudes (and more strikingly evident still further north) that this tenet could not stand serious inspection since some of our local birds build their nests and lay their eggs a few inches above sheets of ice in our muskegs, at temperatures far below those in which they have recently been wintering. It would have been simplicity itself to build aviaries and heat them during the winter months, but for the reason just stated (and others), it seemed waste of time and money, holding not the slightest prospect of success. Temperatures, themselves so variable, could not, in fact, be expected to account for the remarkable regularity of the spring passage. The only observable environmental factor of sufficient constancy at our latitudes appeared to be the annual changes in day-length, which recur with infallible precision and without deviation from year to year.

Botanists have toyed with artificial day-lengths for nearly a century, while already forty years ago Sir E. Sharpy-Schäfer had suggested that the regularity of bird migration might depend on the annual incidence of changes in day-length. The idea was thus not new, although I was at the time unaware of either Schäfer's paper or current work in botanical circles. Such repetition of thinking has often enough occurred in the annals of scientific investigation. Actually Schäfer explicitly states that he thinks the state of the gonads irrelevant; my own contention was the opposite.

The underlying assumption, then, of the initial 1924 undertaking was this; that if the gonads of migratory birds could be brought to spring condition in the fall by subjecting them to lengthening, instead of shortening, days in the autumn, they might turn north when released instead of south.

Juncos conveniently pass through Edmonton in large numbers during September and October. We trapped some of these and divided them between two aviaries made of packing cases and discarded mosquito netting for lack of funds. These stood at the end of our garden, far removed from any sources of extraneous warmth:

if I was going to attempt elimination of the warmth factor, I might just as well do it properly and remove any vestige of doubt. I was probably the only person in Edmonton hoping for low temperatures, but my wishes were amply granted, the minimum to which my birds were exposed being 52 degrees below zero Fahrenheit—84 degrees of frost. In spite of such drastic temperatures, the males of the experimental aviary began to sing during December and were, in fact, developing spring testes. The controls next door, under the shortening days normal to fall, attained a minimal stage of development in October at which they remained stationary through the winter.

My scheme of lighting was as simple as my argument, consisting merely of two fifty-watt electric bulbs in an aviary roughly six feet by six by three. The lights were turned on an hour before sunset, while the birds were still fully awake, and left to burn on the first night till five minutes after dark. Thereafter they were turned on nightly at sunset and left to burn five minutes longer than on the preceding day. Considering that the mornings were still shortening at the rate of about two minutes daily, my birds thus got a net extension of day-length somewhat below the normal spring increase of almost five minutes per day. This was later corrected, while the intensity of the illumination was also somewhat increased. In the lighting as first used, many of the birds retired to secluded corners and fell asleep. By 1928 we had things so adjusted that 90 per cent of our birds showed as steady and regular an increase in gonad development at low temperatures through November and December as the wild birds passing through on migration in spring and subsequently settling in the locality to breed. In 1926 I acquired a large aviary built out of Royal Society of London funds, and about 200 birds.

I propose to omit from this simplified account the side issues arising out of our basic line of procedure in order to present you with a coherent and intelligible picture of the main thread, which still retains its original interest today. Let me repeat again that my source of illumination consisted of ordinary electric light bulbs, devoid of ultra-violet radiation and in actinic value about one ten-thousandth part of summer sunshine. The common denominator as between my experiments and the free environment of the junco was thus entirely a matter of *duration* of illumination, the theoretical assumption on which the whole scheme rested. Considered simply as light, my little bulbs could not even begin to compare with normal sunlight, for they were the merest drop in the bucket of daylight illumination. *Length of day*, on the other hand, was identical under both sets of circumstances.

Why this simple point of logic should be obscure, or otherwise

unacceptable, I personally cannot understand, yet most of the large output of experimental work that has since arisen has attempted to prove that not duration of illumination, but actinic values, are the real clue to the profound physiological changes that are induced in the experimental subjects. This line of thought appears to have been initiated by Bissonnette who was the first biologist to repeat the experiments. He did so in 1930, using starlings, locally abundant in his home town of Hartford, Connecticut. In adopting our scheme of illumination, our own results were fully corroborated, but in repeating another and more revealing undertaking, Bissonnette believed he had, to use his own word, "disproved" my results. I believe this assertion was the starting point of the long series of ingenious investigations that have since followed.

The experiment I allude to was this. In 1928, when we were getting striking developmental uniformity in our illuminated birds, regardless of temperature extremes, it occurred to me that if my argument was really sound, then, if one could devise some way of forcing birds to remain active over a stipulated length of day without the use of light at all, one should get similar results. Various ideas were tried, but none of them worked in *total* darkness for they killed the birds. I therefore ended with a compulsory exercise cage, the perches and gadgets of which were painted white to make them more readily visible and in which I could keep my birds awake without injury with the use of nothing more potent than a single two-candle-power electric light bulb suspended from the remoteness of the ceiling in such manner that no direct rays could penetrate the cage which stood on the floor. Actually this was not far removed from total darkness within the cage (which was roofed), the light being so feeble that the birds did not even attempt to leave their perches as the forcing element crept up on them and compelled them to move; they merely stepped over it.

The cage in which I kept these birds measured three feet by two by one. Controls were similarly housed, in similar lighting, but without the disturbing device. Down the length of the cage ran a central perch, with food and water troughs on the same level. By means of large wheels outside the cage, geared to a motor in such manner that a single revolution was completed in forty seconds, a wooden bar, painted white, entered the cage at the level of the perch, swept slowly along its length, passed out of the cage at the other end, re-entered it on floor level and, after sweeping that, went out again to return once more at perch level. When the birds had been trained to this device, a second bar was added to the belts so that, no matter where a

bird might sit, every twenty seconds it *had* to move. There was not the slightest chance of falling asleep. My philosophical little juncos took their medicine in good part and merely stepped over the advancing bar as it approached. They remained in good health and, more important, showed a rate of testicular development almost precisely identical with our illuminated birds or the wild birds arriving in spring. The controls, in the meantime, slept away the hours of their negligible illumination undisturbed and showed no signs of sexual development.

Bissonnette repeated this experiment with starlings in 1931, using a cage essentially like mine except that it was larger, for his birds were bigger. His results were mainly, though not entirely, negative and, as already pointed out, appear to have inspired much of the work that has since been undertaken. To the best of my knowledge all of it remains inconclusive, if not entirely negative. I regret that there is no chance to review it herewith, but I must point out one item in Bissonnette's undertaking that strikes me as crucial not only to the argument of the moment, but to experimental work involving animals in general.

Starlings are among the most nervous of birds, a fact familiar to every aviculturist who has attempted to keep them in captivity. Bissonnette describes at length how his starlings resisted the treatment by hanging to the wires till so exhausted that they had to seek relief on the perches or floor from time to time. I think I can make the following criticism without being charged with bias for it seems so very obvious. Bissonnette's undertaking cannot be fairly compared with my own for he had introduced a factor (which he himself entirely ignored) that played no part in the case of my juncos since neither nervous disturbance nor muscular fatigue nor physical exhaustion entered into their case at all; as far as it was possible to evaluate them, they remained in a physiologically normal state throughout. But I do not believe this could have been the case with Bissonnette's starlings. From everything known about the mechanism of the nervous system, it is improbable that these negative results can be accepted as significant. They were not even uniformly negative. I mention the point not only for its direct relevancy but because it has entered into other analogous undertakings with animals and is habitually overlooked. In devising such highly artificial experiments, with their many unknowns, as forced exercise in almost total darkness, the nervous responses of the subjects are of equal importance with anything else that may enter into the adopted scheme. I think possibly more so because they are basic; everything else depends on them.

In 1936 during the course of a year in London, when chancing to go to a theatre in December, I noticed hundreds of starlings roosting on various buildings of the West End. These birds flock in from the surrounding country nightly and voluntarily subject themselves to the noises and lights of the city, twittering away intermittently till the theatre crowds have gone home and peace settles at length on the West End. It occurred to me that here was Bissonnette's experiment with starlings being carried out on a magnificent scale with irritation of the nervous system eliminated, for the birds had chosen these surroundings of their own accord; they find them congenial, not disturbing. I decided to collect samples during January. I thought that would be simple, but the case proved otherwise. After much difficulty, and with the assistance of various friends, I finally ended up by securing a dozen birds off the face of a well-known institution in the West End by shooting them from the roof with a shotgun at midnight. Together with James Fisher (then of the London Zoo) who was with me, we adjourned, having successfully evaded the police, at about 2 A.M. to the lab at University College where we opened our birds and fixed their gonads. These were in a high state of development, roughly two months ahead of their country cousins of the same date. In fact it looked as though starlings disturbed at night, in very feeble lighting, and when not temperamentally upset, *did* react precisely like my complacent little juncos.

My friend Dr. Bullough, of McGill, thinks he has a better explanation of these facts; I look forward with much interest to the day when he produces the proof.

The war appears to have offered an opportunity for a final answer to this question, a chance that nobody managed to take, but it was not for lack of effort. With the complete blackout to which London was subjected during the war obtaining for several years, it seems regrettable that starling samples were not then taken, when even the dim lighting of London's streets was eliminated and its starlings were kept awake by disturbance alone.

An unexpected bit of support came later from Iowa, where Dr. Witchi long ago interested himself in certain aspects of this debate. He made the interesting discovery that sparrows roosting in total darkness in barns along Iowa's motor highways with their incessant traffic disturbance, show premature spring development of their gonads as against sparrows roosting in exactly similar barns in the same locality on secluded farmyards.

Before leaving this aspect of the case, I would like to add a comment. In the many papers that have now appeared on the topic,

the authors have confined their attention to the effects of lighting on the gonads and, of course, on the pituitary, on the activities of which the seasonal fluctuations of the gonads directly depend. But it seems to me that that is only part of the story, because the entire physiology of the animal must inevitably be involved. The marked changes induced in pituitary and gonadal activity are surely but tangible symptoms of a more general and deeper seated effect. I would like to suggest that if this viewpoint is correct, the day may come when a prescribed adherence to days and nights of certain relative durations may be adopted as a recognized therapeutic measure in dealing with certain classes of nervous disorder, or may have other applications.

To revert now to the migration side of the experiments. When still using juncos, I released many of my birds in known states of gonadal development to see what they would do with themselves. To condense a long story, let me merely state that a percentage of our experimental juncos disappeared upon liberation, while of the controls, with their gonads at the winter minimum, we lost none; true, a few disappeared into cats and shrikes, for some of them would hang about the garden for days before wandering into our traps, but they were nevertheless 100 per cent sedentary. Weather played a marked part in the responses of our experimentals, severe conditions inducing prompt return to the traps which were always kept open and baited. Whether the departees went north, south, east, or west we could not tell and it became apparent that we would have to find better guinea-pigs of the airways, birds universally familiar and large enough to be followed on release, and unprotected by law so that we could legally ask the world at large to collect them for us. The obvious answer was the crow, omnivorous and easy to keep, avian vermin that might be shot by anyone at any time, and a species that migrates in Alberta with great regularity. A vast majority of Canadian prairie crows winter in Oklahoma or Kansas, while the fly-line from Alberta is apparently direct.

Two main difficulties presented themselves; the first was to get the crows. We found the reputed wariness of the species not in the least exaggerated but finally developed a technique that produced results and in 1931 we had some 500 crows available for our biggest undertaking. While I admit that it was hard labour, with long days and short nights, it was at the same time both informative and entertaining. We certainly learnt a lot about the psychology of crows.

The second difficulty was to get sufficient publicity for our birds to ensure profitable returns. When you let crows loose to high heaven,

only a widespread effort can hope to get them back, and it has to be an *effort*. Newspapers and radios were generous in their assistance, but the preliminary campaigning had inevitably to be incessant and unrelenting. This also was hard work. When I state the fact that, on the average, we recovered about 60 per cent of our birds—100 per cent on occasional small groups—you will appreciate something of the extent of the preliminary spade-work. Over a thousand miles separated our northernmost and southernmost recoveries.

Our scheme of operations was simple. Having trapped our crows we shipped them into Edmonton and released them in large flying cages, illuminated on the same basis as our juncos. The experimental cage was 100 feet long, lit with twenty-five 500-watt standard electric light bulbs. Food consisted of all the rotten eggs that Edmonton candlers could produce, the entire output of dogs and cats from the city pound, an occasional horse, hundreds of pounds of fish, cabbages, stale loaves, buttermilk, etc. On this varied diet they flourished. When we finally liberated them with tails resplendent with the most brilliant yellow Duco obtainable (for ready recognition—a mode of marking that has since been widely adopted in Europe) they were crows to be proud of.

They received the same light schedule as the juncos, a small aviary of these birds being housed inside the crow cage as an incidental check. Both the 1929 and 1931 birds were turned out during November, long after the last wild crow had gone south. We liberated controls and experimentals together on both occasions, trusting to the birds to sort themselves out according to their own inclinations although, owing to the gregarious habits of the species, this seemed to be taking a distinct chance. Since it added something to the stringency of the test, however, the idea was adopted. On a theoretical basis we might expect the experimentals to travel north-west, the spring direction, and the controls either to remain sedentary or to turn south-east, the fall direction.

Edmonton is situated on the fringe of the northern wilderness. One hundred and twenty miles to the north-west is Lesser Slave Lake with enormous expanses of muskeg and forest intervening between the city and the lake. Should our experimentals actually go that way, our chances of recovery were virtually nil. In 1929 we liberated our birds from Edmonton. While we got several returns from the north-west within fifty miles of town, our two most notable recoveries, at something over 100 miles north-west, actually came out of this wilderness and were patently the merest flukes. One was retaken by a squatter's wife, aware of the experiments and hoping for one of our

cash prizes, on an isolated patch across the Athabasca River, while the other came from a trapper without a radio, ignorant of what was afoot, and who shot the bird with a big-game rifle merely to satisfy himself that he was still sane. The sight of a mid-winter crow among the ravens around camp put him into a state of mental jitters which, fortunately for us, he decided to liquidate by collecting the bird. On picking it up, he discovered our band and so finally, months later, when he came to town, we got the record. In the meantime two substantial flocks of crows had turned up on the shores of Lesser Slave Lake, another 100 miles further on, the last previous crow having been seen there two months before. The birds made their appearance nine days after our releases. We offered as much as \$25 reward for a single sample, but in spite of much local effort, none was procured. Of our experimental birds, 50 per cent were a total loss, completely unaccounted for; of our controls, only two out of fourteen escaped us (about 15 per cent). Such of them as went south passed over well-settled country, amply patrolled. Some of our experimental birds also went south; I have no doubt that the majority of these were among those retaken.

In 1931 we decided to liberate our birds 250 miles south-east of Edmonton so that the experimentals would have some 300 miles of settled country ahead of them as they travelled north-west. The aviaries were still located in Edmonton and the intention was to fly the birds down to Medicine Hat in south-eastern Alberta by plane and there turn them out. Through circumstances beyond our control, we came down at less than half the distance at a small place called Hackett and sixty miles west of Hackett once again began the limitless wilderness. Once more we lost over 50 per cent of our illuminated (north-bound) group, but less than 30 per cent of the rest. That difference (about 25 per cent) is surely significant, the more so since the experimentals only were branded with yellow tails and for that reason alone should have given us higher returns than all the other groups together. Moreover, the experimentals outnumbered all the rest. Yet the opposite was the case.

To summarize at this point: north-bound birds came entirely from the illuminated groups and to this there were no exceptions. All other groups, to which I will refer further in a moment, either did not travel at all or went south. Our chances of recovery from the north-bound birds were on both occasions diminished by the natural features of the Province, and on both occasions we lost a high percentage of them. Our showing on south-bound groups was vastly better. This is, of course, merely circumstantial evidence, but without straining

my conscience to boost results in our favour, I think there is only one logical interpretation to this aspect of the case—that we actually did much better than the returns indicated. Whether that is so or not, the fact remains that a limited reversal of the fall migration had been successfully induced in a percentage of our birds. Time does not permit me to outline the theoretical implications of these results, but they are far-reaching.

I think we can in part account for the irregularity that our north-bound individuals showed. Unlike the juncos, in which quite extraordinary uniformity could be obtained in the matter of testicular recrudescence under the stimulus of artificial light at low temperatures, our illuminated crows showed much disparity in their responses. There are two possibly good reasons for this: first, the varying temperaments of the crows themselves, some of which were bold and others shy, some as complacent as the juncos and others extremely nervous; or, secondly, there was the fact that our source of light was devoid of ultra-violet radiation. There is no obvious reason why crows should trouble to migrate south from Alberta. My liberated birds not only stood the climate as well as the resident ravens and magpies, but did themselves equally well in the matter of food; they were all in flourishing condition when recovered. Yet actually they go a long way south, far enough to stay in touch with a constant supply of solar ultra-violet, possibly, with crows, a physiological necessity throughout the year. This can hardly be the case with related species resident in Alberta, for the winter ultra-violet supply at Edmonton latitudes is negligible; the same may apply to juncos, which do not go as far south as crows. If this were the case, a source of illumination devoid of ultra-violet might meet all the essential requirements of the junco, and give us the striking uniformity which we actually obtained but might fail in the case of crows. Certainly, when we next repeat these experiments the source of illumination will be amended in the matter of ultra-violet deficiency.

In 1931 we had all the crows we could use. As with our juncos in 1927-8, we ran subsidiary experimental groups concurrently with the main series and certain of these I must briefly allude to. First, there were the castrates. In theorizing during the early days, it was assumed that if the northward passage depended directly on the developmental state of the gonads, so should the southward, although this would not *necessarily* be so. In 1931 we had several groups of capons receiving different treatments as a test of both hypotheses. As far as the southward trek is concerned, it can be certainly stated that the fall condition of the gonads is not concerned in the story, for,

our castrates proceeded south as fast and as directly as intact individuals. Subjecting these birds to illumination proved, as expected, to be quite ineffective for, with a single exception, none of them turned north. This exceptional bird was perhaps the most interesting single individual of the series.

During the process of castration a single testis of one bird dropped off the spoon at the moment of extraction and fell back into the body cavity. The episode was fortunately recorded on this bird's case-card. He was recovered about forty miles north-west of Hackett but not till February (which allows for mere wandering) and the body was not sent in. It seems possible that the testis may have successfully grafted itself, in which case the bird would actually belong to our illuminated experimentals with one testis still functioning as a centre of hormone production. These illuminated castrates also indicated that stimulation of the pituitary by itself is ineffective; gonads activated by the pituitary appear to be the seat of migratory motivation, at least as one must interpret the evidence at the moment.

We injected another group of capons, unlit, with testicular extract hoping, by this means, to get corroborative evidence in the matter of gonadal hormones. Inevitably we had to guess the dose, and decided on five bird units, 0.5 cc. daily, subcutaneously injected. Fifteen birds were involved and of these eleven were recovered, i.e., 73 per cent. We got them back over a period of weeks. The interesting thing about them was that they proved completely sedentary. They were retaken from all directions, mere wanderers, and within short distances of the point of liberation. The southward impulse was evidently killed and it seems at least possible that with stronger doses we might have induced movement to the north. Another small group of sedentary birds, unoperated individuals (with a recovery rate of 80 per cent), was produced with the use of a gonadotropic (pituitary) extract. None of these apparently attempted to go south either. Stronger doses might again have been more fruitful. These and other results all suggest obvious lines of attack in some repetition of the future.

In conclusion, may I briefly refer to two other ventures. The normal migratory route of crows plying between their breeding grounds in Alberta and their wintering grounds in Oklahoma (and *vice versa*) is fully known through large-scale banding operations by the U.S. Biological Survey and others in the south, and through our more limited contributions from the north. The directions that my birds have always taken have conformed strictly with this crow tradition. Migration, like homing, is generally supposed to depend

on previous experience, and on known landmarks and memory, an argument, however, that palpably fails in the cases of many species when critically examined. With special reference to the crow this supposition was tested in two different ways. In 1935 some eighty Alberta-caught crows were shipped to Portage la Prairie in Manitoba, 720 miles east of Edmonton and a little south. The laborious campaigning that had prepared the field for local liberation in Alberta had proved impossible by remote control in Manitoba and only three significant returns were obtained. These individuals had, however, adhered to their habitual south-east fly-line. Had they kept going along this road, roughly paralleling their customary Alberta direction, they would have ended somewhere in the state of New York instead of Oklahoma.

These few returns are insignificant in themselves, but become suggestive in light of the fact that, in East Prussia, between 1935 and 1939, similar experiments were carried out with the local hooded crow, whose breeding and wintering grounds are as well known as those of our own birds. They were transported from five to seven hundred miles west and then liberated; the results were precisely like ours. One hundred and seventy-six birds were recovered over these years, representing 20 per cent of the original number used. All but eleven of them had, like our three recoveries, paralleled their habitual direction, and settled down at spots for the winter about as far west from where they should normally have been as the original distance of transportation westward at the northern end.

Finally, in 1940, on November 9, we turned out fifty-four young birds of the year at Tofield, Alberta, the point at which they had been trapped during late summer. Except that they had been sumptuously fed on rotten eggs and cats in Edmonton during the interval, nothing else had happened to them; they were merely retained till all wild crows had gone south and then turned loose without any adults. The outcome was striking. Some 60 per cent were recovered; of these not a single bird had deviated significantly from the standard fall direction, while some of them, in travelling fifty miles per day, had broken all previous speed records. The mean line of travel for all of them, when projected on the map, terminated in the centre of Oklahoma. These youngsters knew nothing of landmarks, geography, or topography beyond the district in which they had been hatched and where they were again released. They presumably knew nothing of the history or habits of their race, or the significance of north and south, or of the existence of Oklahoma, or anything else that might provide a clue as to where to go or how to get there. In spite of all

that, they took the unknown road to their unknown destination with unerring precision.

It seems to me that one is compelled to describe this performance as an *inherited sense of direction*, whether one likes this habitually spurned definition or not, or whether it means anything or nothing in our present state of knowledge. The establishment of simple facts of this sort, that have in the past been considered untenable myths, seems to me to be one of the most useful functions of experimental biology. Facts will always be facts, concrete and tangible, and they will undoubtedly always give rise to further theories, man being what he is, the most inquisitive of all animals. Out of such theories there arises that further type of experimental work that sifts and analyses, that probes into causes and effects, that eliminates and establishes, and which, if one happens to be lucky, may bring to light some unexpected generalization that may further our understanding of life at large.

THE INFLUENCE OF SESAME OIL ON THE GROWTH
RESPONSE TO TESTOSTERONE PROPIONATE
(T.P.) IN RATS

By FERNAND SEGUIN

Presented by GEORGES PRÉFONTAINE, F.R.S.C.

INTRODUCTION

FOR intra-muscular or subcutaneous injections, testosterone propionate and many other steroid hormones are usually dissolved in sesame oil. Available evidence seems to prove that this vehicle is not physiologically inert (Bruce, 1940; Clauson, 1940; Pollia, 1937; Stein, 1943). For instance, it may influence the rate of survival after adrenalectomy (Spurr, 1939; Tobin, 1941) or inhibit the action of small doses of androsterone (Crafts, 1942).

We decided to investigate the effect of sesame oil on the growth response in rats, either normal or castrated, following androgenic treatment.

MATERIAL AND METHODS

Ninety Ayerst male albino rats were used in this study. Half were castrated at 37 to 38 days of age. This group and an equal number of normal rats were further divided into three sub-groups, each comprising fifteen animals. The first was kept as control, the second received daily subcutaneous injections of sesame oil, and the last was given daily subcutaneous injections of 0.05 mg. testosterone propionate (T.P.) in sesame oil. No injections were given on Sundays.

During the course of the investigation, which lasted 43 days, the animals were kept in individual cages and were fed Purina Fox Chow and water *ad libitum*.

Body weights were taken at the time of the operation and weekly afterwards until the end of the experiment. For comparison purposes, the results were treated statistically; the difference between two means was considered "statistically significant" when it was at least four times its probable error.

TABLE I

Rat Group	Number of Rats at the End of the Experiment	Initial Weight (in grams)	Final Weight (in grams)	Per Cent Increase in Weight
Control castrates	14 ¹	110.7 \pm 2.6 ²	235.4 \pm 4.1	112.6
Sesame oil treated castrates	15	111.8 \pm 2.7	228.6 \pm 5.8	104.5
Hormone treated castrates	14 ¹	110.4 \pm 2.6	244.9 \pm 4.9	121.9
Control normals	15	110.1 \pm 2.3	267.9 \pm 4.3	143.3
Sesame oil treated normals	14 ¹	113.4 \pm 3.1	255.8 \pm 5.2	125.5
Hormone treated normals	15	111.5 \pm 1.8	250.9 \pm 4.3	125.0

¹One animal died during the experiment.

²Probable error of the mean.

RESULTS AND DISCUSSION

As seen from Table I, initial weights of all groups were equal for practical purposes. At the end of the experiment, normal rats were significantly heavier than the castrates, in confirmation of previous findings (Donaldson, 1911; Evans, 1927; Korenchevsky, 1930, 1936; Lawless, 1938; Rubinstein, 1939a; Weil, 1941).

Hormone treated castrates show a small weight increase above the control group which is in accordance with results obtained by other investigators (Korenchevsky, 1936; Rubinstein, 1941; Weil, 1941), but the treatment of normal rats with T.P. inhibits the body growth, although in a non-significant fashion. The literature dealing with the effect of T.P. on the growth of normal rats is contradictory, some authors finding a depression (Korenchevsky, 1937; Rubinstein, 1939b, 1944), while others note an enhancement of growth or no effect at all (Rubinstein, 1940a; Shay, 1941; Turner, 1941). Hormonal dosage or seasonal variations (Rubinstein, 1940b) might play a part and other factors might also be involved. For instance, if one considers the

influence of sesame oil alone (see Table I), one sees that it inhibits the growth curves of either normal or castrated rats. The effect, although not very pronounced, could perhaps explain why hormone treated castrates fail to attain the normal growth level. It could also explain why our normal rats, when treated with T.P., show a slight impairment of growth. Indeed, the percentage increase in weight for sesame oil treated animals is lower than the increase for any of the other groups. It is reasonable to assume that if it were not for sesame oil, both hormone treated groups would be heavier and that the hormone treated normal rats would be at least as heavy as the normal group.

CONCLUSION

Sesame oil, when administered alone to normal or castrated rats, causes an impairment of growth which, in the case of normal rats, is equivalent to that produced by treatment with testosterone propionate dissolved in sesame oil. These results would seem to indicate that sesame oil is not a physiologically inert vehicle and that experiments conducted with substances dissolved in that medium should be carefully interpreted.

ACKNOWLEDGMENTS

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second line: *for* plasma-Monographien, 5:19, *read* plasma
Monographien, vol. 19. Gebruder Borntraeger, Berlin.

third line: *for* physiology *read* physiologie.

THE PERMEABILITY OF CELLS UNDERGOING NUCLEAR DIVISION

By HERBERT STERN

Presented by GEO. W. SCARTH, F.R.S.C.

INTRODUCTION

THE permeability of dividing cells has long been studied, especially that of fertilized marine and fresh-water eggs. Unfortunately, to the question of correlation between degree of permeability and mitotic activity these studies have yielded equivocal answers, and consequently no generally acceptable conclusion has yet emerged (Fauré-Fremiet, 1925; Heilbrunn, 1937). Correspondingly, opinion is divided regarding the possible significance of this property to the physiology of cells undergoing division. Some reviews of nuclear division, including the comprehensive one by Schrader (1944), consider the subject briefly, if at all; others, like that of Bujard (1941), devote considerable attention to the problem. From an *a priori* standpoint there is no reason, of course, why changes in permeability should be associated with nuclear division, but the apparent importance of the plasma-membrane in the life history of most cells and the permeability changes which many plant cells have been shown to undergo in various physiological adaptations—resistance to frost, drought, and fungal infections—suggest by analogy that some relation probably does exist between cell permeability and nuclear division.

The lack of uniformity in results of previous investigations might be attributed to at least two conditions—the comparative rapidity of mitosis in most plant or animal cells and the kind of environment to which dividing cells amenable to permeability studies are exposed. Generally, the duration of a complete mitotic cycle in either plants or animals varies from one-half to three hours depending, of course, upon the prevailing temperature (Sharp, 1934). Thus, morphological changes occur in fairly rapid succession and probably permeability changes, if they are associated with nuclear division, behave in much the same way. Under such conditions the detection of changes in permeability would be difficult, especially in these tissues where only a fraction of the cells are dividing at any one time. The fertilized eggs of many marine and fresh-water animals may be cited as an exception, for the permeability of these cells when dividing appears to be readily determined. Here, however, a second condition

should be considered, namely, the open environment to which the cells are exposed. A very large number of proliferating tissues are contained in a closed organic environment—with one of these, incidentally, we are here concerned—and it seems reasonable to expect differences in the permeability of cells bathed in organic fluids many of which play an important role in the stimulation and metabolism of nuclear division, and those exposed to an environment, largely inorganic, from which relatively few substances are required.

One group of cells satisfying the requirements for a more facile study of permeability are the pollen mother-cells and pollen-cells of the red Trillium (*T. erectum*). Individual cells are about forty microns in diameter and lend themselves readily to investigation by the plasmolytic technique. The cells in a single anther are to some extent homogeneous with respect to stage of division and there is no difficulty obtaining preparations in which all cells are undergoing or about to undergo nuclear division. Furthermore, their rate of division is very slow if the buds are stored at about 2° C., meiosis of the pollen mother-cells extending for several months, and mitosis of the pollen-cells extending for at least several days.

A full report on the protoplasmic studies of these cells will appear elsewhere (Stern, 1946b). Here, the outstanding changes in permeability which have been observed are briefly described.

RESULTS

Consider first the pollen-cells of Trillium. Even before the onset of active mitosis their permeability to polar solutes is comparatively high. A commonly used plasmolytic agent such as urea illustrates the point. In a twice isotonic solution 90 to 100 per cent of the cells deplasmolyze in times varying from 4 to 15 minutes. In most plant cells, on the other hand, when they are thus initially plasmolyzed to half volume, deplasmolysis time for 50 per cent of the cells varies from 15 minutes to several hours. The cortical cells of *Catalpa*, for example, require 15 to 30 minutes if hardened to frost and 1 to 2 hours if unhardened (Levitt and Scarth, 1936). Even without a comparison of absolute permeability values (such a comparison would favour the pollen-cells since the cells are large and spherical and have therefore the lower surface/volume ratio), it is apparent that the permeability of pre-mitotic pollen-cells appreciably exceeds that of *Catalpa* cortical cells—and probably, of most other plant cells.

More spectacular, however, are the permeability changes which occur during active mitosis. Non-dividing pollen-cells, although

permeable to substances of low molecular weight such as urea, are poorly permeable to glucose and virtually impermeable to sucrose. In this respect they resemble most plant or animal cells. But, with the onset of active division, or immediately preceding it, the pollen-cells become increasingly permeable to these two solutes. Ninety to 100 per cent deplasmolysis time in a 1.5 M sucrose solution drops from 6 hours at the very beginning of division, to 4 hours in early prophase, to $1\frac{1}{4}$ hours in late prophase, and to zero to 10 minutes in very late prophase or early metaphase. For technical reasons absolute determinations of permeability have not been made. Nor have changes

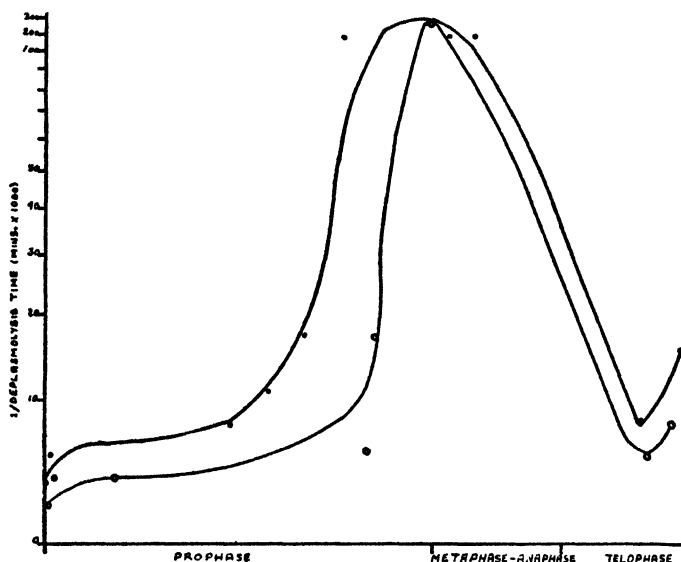


FIGURE 1.—Permeability changes during mitosis of *Trillium* pollen-cells. Dots represent the reciprocal of times for deplasmolysis in a 1.0 M sucrose solution and circles that in a 1.5 M solution. The stages of division at which the values plotted occur are only roughly approximated. (Adapted from Stern, 1946b.)

in osmotic pressure been taken into account, but such changes are too small to affect the trend significantly (Stern, 1946b). It may be inferred from results plotted in Figure 1 that the degree of permeability reaches a maximum value somewhere near the end of prophase or in early metaphase. Beyond this, permeability declines with the development of the mitotic cycle, although there is evidence of a second rise at telophase. Data are lacking, unfortunately, for a more complete description of the permeability changes, and evidence is needed to locate these changes more precisely in terms of mitotic

stage—the present curves are merely crude representations. It is interesting, however, that in his estimations of permeability in fertilized *Arbacia* eggs, Herlant found maximum permeability to occur at prophase and a second though smaller maximum to occur at telophase (Fauré-Fremiet, 1925).

The sequence of permeability changes in the meiotic pollen mother-cells differs markedly, in at least one respect, from that in the mitotic pollen-cells. In the former, long before nuclear division is morphologically apparent, the cells show an exceptionally high permeability to polar solutes. The so-called pre-leptotene cells do not even plasmolyze in a 1.5 volume molar solution of sucrose. This behaviour is clearly not due to a high intra-cellular osmotic pressure, for there is no corresponding increase in cell volume with dilution of the medium. The absence of plasmolysis would appear to be due to an extremely rapid penetration of sucrose so that equalization of osmotic pressure between cell-sap and medium is effected as quickly, or nearly so, as the passage of water. This calls for a very low resistance of the plasma-membrane to solute movement and if further proof be required, it may be found in the fact that pre-leptotene pollen mother-cells when placed in distilled water, do not swell but become highly refractive and generally coagulate owing, presumably, to the outward diffusion of sap solutes. Even the acidic sulfonothalein dyes, which generally do not penetrate living cells, enter these cells freely although less rapidly than does sucrose. So do polyvalent cations, to judge from the effects of low concentrations of their salts (0.005 M-0.0001 M) on the refractivity of pollen mother-cell protoplasm. That these cations have not been observed to antagonize their own entry or that of sucrose, as commonly occurs in plant cells, is further demonstration of the prevailing high permeability.

With the onset of active meiosis there is a drop in degree of permeability. In many suspensions of pollen mother-cells undergoing division, a fraction of the cells plasmolyze in hypertonic sucrose concentrations, the magnitude of the fraction depending on the proportion of meiotic stages in the suspension. But precise location of the permeability changes in terms of the meiotic cycle is complicated by various factors and because of the exploratory nature of the work no such location was attempted. It is clear, however, that following the completion of meiosis, there is a return of the cells to a condition of true semi-permeability, at least with respect to sucrose.

DISCUSSION

Four questions may be raised for discussion in connection with the permeability changes described:

- (1) What is the *nature* of these changes—active or passive?
- (2) How *general* are they in the biological kingdom?
- (3) What is their *significance*?
- (4) What is the *mechanism*?

1. The association of a special degree of permeability with cells undergoing division suggests a comparison of the writer's results with those of the schools of Hoagland and Steward on the uptake of solutes by growing tissues. It now appears certain that rapidly growing tissues absorb salts in relatively large amount from their external growth medium, even against a concentration gradient, and that the capacity thus to accumulate salts is due wholly to those cells in the tissue which are dividing or growing. Furthermore, it is established that the accumulation is not linked to a high passive permeability of the membrane for, among other things, when such tissues are placed in aerated distilled water there is no exosmosis of solute. The fairly rapid intake of solute is presumably not governed by any physical property of the plasma-membrane, but by an inward energy gradient created by prevailing metabolic conditions. It is generally agreed, in fact, that one concomitant of salt uptake is a vigorous carbohydrate metabolism, but beyond that opinions vary as to the more immediate causes of solute penetration. Brooks (1941) suggests organic-inorganic ion exchange; Steward links the uptake to protein synthesis (1937). The essential feature, irrespective of mechanism, is the movement of solute against a gradient of concentration under conditions of "active permeability" in contrast to solute movement with the concentration gradient under conditions of "passive permeability."

The question should therefore be asked as to whether the observed deplasmolysis or lack of plasmolysis in pollen-cells and pollen mother-cells at certain stages of division is due to an extraordinarily active uptake of solute, or to a special condition of the plasma-membrane which renders it highly permeable to the solutes in question. In the writer's opinion the evidence points conclusively, and obviously, to the latter interpretation. It would be a tenuous explanation indeed that postulates an active uptake of sucrose by vigorously metabolizing cells which do not simultaneously utilize either the sucrose absorbed or its equivalent previously present. Yet in no other way, presuming an active uptake, could the increase in osmotic pressure necessary to produce deplasmolysis be explained. It is equally difficult to conceive

of so rapid an active uptake as would be required in the case of pre-leptotene pollen mother-cells suspended in a 1.5 M solution. Nor could the observed order of penetration from iso-osmotic solutions of urea, glucose, and sucrose—easily explained in terms of a passively permeable membrane—be accounted for by an active accumulation. Furthermore, in contrast to the behaviour of tissues known to be actively accumulating solute, pollen mother-cells suspended in distilled water coagulate rapidly, thereby strongly pointing to a quick exosmosis of intra-cellular solutes through a highly permeable plasma-membrane. Thus, the point of view here adopted is that in these dividing cells, quite apart from their capacity to accumulate solutes in the course of growth, there is a temporary change in composition of the plasma-membrane such that the cells become less resistant to the passage of solutes, inward or outward. That is, the changes observed are changes in degree of passive permeability.

2. It may then be asked whether the phenomenon is unique for the spermatogonial cells of *Trillium erectum*, or if it differs only in degree from cell division in general. Evidence is available from some preliminary observations by the writer that changes similar to those found in the red *Trillium* occur in the pollen mother-cells of *T. grandiflorum* and *Tradescantia reflexa*. There is also, in addition to the results on animal eggs, some indirect evidence that increases in permeability occur in many plant cells undergoing division (Stern, 1946b). The recent report on the biological action of the mustards (Gilman and Philips, 1946) suggests the same conclusion. From this report it appears that when threshold concentrations of mustards are applied, only those tissues are affected which normally exhibit high rates of proliferation and growth. It is not improbable—although alternative explanations are equally possible—that the higher susceptibility to mustards of proliferating tissues is due to the higher permeability of the dividing cells. In nearly all of the above cases, however, the highest degree of permeability, observed or inferred, does not equal in magnitude that found in *Trillium*; generally it appears to be much less. Obviously then, a very high degree of permeability is not a characteristic shared by all cells undergoing nuclear division and in terms of absolute permeability values no generalizations can be made. On the other hand, with respect to *variations* in permeability, the behaviour appears to be more general, and it seems justifiable to suggest an increase in degree of passive permeability as a feature of most plant or animal cells undergoing division. The fact that the pollen mother-cells completely lose during one phase of the division

cycle their property of semi-permeability would thus be associated with the special conditions obtaining in the anther rather than with physiology of cells in division.

3. If so, of what significance are these changes to nuclear division? Are they useful *per se* in division or are they merely an incidental result of more deep-seated changes which are part of the mechanism? Certainly a possible use in all types of cells is difficult to visualize. To cells which divide in an inorganic environment a high passive permeability would be more an evil than a blessing, since the retention within the cells of diffusible solutes, if at all possible, would require an otherwise unnecessary expenditure of energy. Perhaps cells contained in an organic medium, as are the spermatogonial cells of Trillium, might profit from an increased permeability. Conceivably, the penetration into such dividing cells of substances required for division would require less work because of the higher membrane permeability. From the standpoint of energetics, this could be regarded as an advantage. But the more permeable the cells, the more necessary does it become that cell-sap and medium be similar in composition. The benefits of an increased permeability must therefore be limited by the media in which the cells divide and since many cells multiply in media unlike their own sap in composition, the usefulness, if any, of the changes can hardly be general.

In this connection, however, secondary effects may be of some importance. The penetration into dividing cells of substances to which mature cells are impermeable would result in a higher susceptibility of proliferating tissues to these agents than that of non-proliferating ones. The possibility has already been referred to in the case of the mustards. The fact that mustards have been reported to produce mutations adds an importance to the relation from the standpoint of genetics.

4. Probably, the true significance of the changes in permeability lies in the fact that they reflect deeper-seated processes which are part of the mechanism of division. Even in cells less permeable than the pollen mother-cells of Trillium, an increase in degree of permeability upon hardening to frost appears to be associated with large changes in the protein and lipid fractions of the cells (Siminovitch, unpublished). Suggestive evidence in the pollen mother-cells is not lacking. During division, the protoplasm shows varying degrees of lability and this may well be attributed to corresponding variations in properties of the protein substrate (Stern, 1946b). The cell surface too, in the course of division, shows changes in property which may not be unrelated to the lipid component of the protoplasm

(Stern, 1946a). But these phenomena require a more complete investigation and probably in their resolution—to be achieved chiefly by chemical studies—the significance of permeability changes will become apparent.

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